# BIOACTIVE CHEMICALS FROM MARINE ORGANISMS - EXTRACTION AND ANALYSIS

A thesis submitted to

Madurai Kamaraj University for the Degree of

**DOCTOR OF PHILOSOPHY IN CHEMISTRY**



By

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**Dedicated to**
**my beloved wife, Rani**
**my daughter, Rini**
**and**
**my son, Dimpu**

## DECLARATION

I hereby declare that the research work described in this thesis **"Bioactive chemicals from marine organisms - extraction and analysis"** has been originally and independently carried out by me under the guidance of **Prof S Sivasubramanian** and **Prof S Muthusubramanian** in the Department of Organic Chemistry, School of Chemistry, Madurai Kamaraj University, Madurai. This work has not been submitted elsewhere for any other degree or diploma.

**(I RAJENDRAN)**

## CERTIFICATE

This is to certify that the work described in this thesis **"Bioactive chemicals from marine organisms – Extraction and analysis"** has been originally and independently carried out by **Mr I RAJENDRAN** under our guidance. This work has not been submitted elsewhere for any other degree or diploma.

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Finally I wish to express my deepest gratitude and appreciation to my family members for their constant encouragement throughout my academic career.

**I RAJENDRAN**

## CONTENTS

## Preface

The main aim of the present investigation is to analyze the different sponges found in the Gulf of Mannar and Palk Bay. A brief introduction regarding the different sponges available and the various organic compounds isolated from them are being listed out in the introductory chapter. A note on the experimental details is provided in chapter 2.

The systematic phylochemical studies on five different sponges, *Siphonochalina* spp., *Cervicornia* spp., *Hippospongia* spp., *Hyrtios* spp. and *Spongia* spp. collected from the Palk Bay of Mandapam and Rameswaram island areas have been carried out and the results are presented in chapters 3, 4, 5, 6 and 7 respectively.

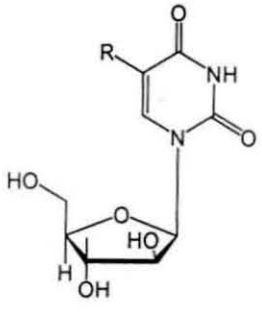
The extraction and chemical analysis of one type of fin fishes, *Tetraodon* spp. found in the Palk Bay were done and the results are presented in chapter 8.

Realizing the presence of several compounds having five membered heterocycle with sulphur/nitrogen among the marine natural products, it was planned to synthesise some new organic compounds having two five membered rings containing both sulphur and nitrogen as ring atoms. Thus several thiazolidinones have been synthesized, characterized and tested for their antifungal behaviour. The results are presented in chapter 9.

The oceans and sea constitute more than 70% of the earth's surface. The biologically diverse and ecologically complex marine kingdom comprising both flora and fauna may, therefore, be considered as the largest reservoir of natural products on earth. But this rich source has become the subject of systematic investigation for the benefit of human race only around the beginning of the twentieth century. This is in contrast to the terrestrial natural products, which have been exploited almost. With the help of self-contained underwater breathing apparatus (SCUBA), it became possible to go down to more than 200 feet below the sea level.

Surprisingly, in contrast to the notable development made in the field of marine products around the world, the exploration of the Indian Ocean and its two arms extending in the north - the Arabian sea and Bay of Bengal has started only in the late seventies.

Marine natural products (MNP) chemistry has come a long proliferating way since the pioneering work of Bergmann about 50 years back[1] when his isolation of spongothymidine and spongouridine from the Caribbean sponge *Tethya crypta* paved way to the development of new pharmacologically important compounds. There is great accumulation of literature on MNP based on the compound types as well as the phylum.[2] Since 1970, chemists have been unraveling the fine structures of these organic compounds often termed as the secondary metabolites produced by marine organisms and the reports have been reviewed by Faulkner.[3,4]




**R = H, Spongouridine**
**R = $CH_3$, Spongothymidine**

It is intriguing to find new compounds arising out of dramatically different marine environment by biosynthetic pathway with new building blocks incorporating unprecedented enzymatic reactions. The novelty of these molecules has attracted the

synthetic chemists to target new analogues and new synthetic methodologies.[4k] Though they appear for chemist as a series of new organic compounds, they serve as principles between marine organisms for their effective communication in the marine eco system in the form of *allomones* (chemicals benefiting the producing organism eg. repellents), *kairamones* (benefiting the receiving organism eg. toxin producing bacteria) and *pheromones* (used for intraspecific communication) for their mutual benefits.[4l,m] They are group specific, co-existent and co-evolution of species.[4n,o]

There has been increased interest in this established new field of multidisciplinary nature by chemists, biologists and pharmacologists as evidenced by the appearance of increased number of patents on methods of isolation and application of natural products, especially of marine origin and periodic review publications in the past few decades. Marine environment accommodates more than 80 percent of earth's phyla. Marine plants, animals and microorganisms exhibit processes and produce substances unknown in terrestrial organisms. Unique chemical structures, unusually high biological activity and participation in intra and inter specific relationships in underwater communities are the reasons for great attention to these substances.

The potential economic and public health benefits of pharmaceuticals, pesticides, hormones, enzymes and polymers derived from marine organisms are high, yet under exploited. The development of novel products from the sea has the potential to greatly contribute to new treatment for human diseases such as cancer, AIDS, inflammation diseases, etc. by eliminating drug resistance. Some of the compounds of significant importance in terms of their antitumor, cytotoxic, antiviral, antiparasitic, antimicrobial, receptor-antagonistic, anticancer, anti-AIDS, antibiotic and enzyme inhibition activities are in clinical trials.[5]

With the development in the taxonomy of marine organisms (*eg.*Indian sponge)[6] and modern powerful spectroscopic techniques like $^{1}H$ & $^{13}C$ NMR, 2D NMR, HRMS, and single crystal X-ray diffraction analysis, endless parade of novel structures have been appearing in the specialized journals. This situation hardly relies on the older methods of chemical degradations or correlations on the minute quantity of compounds isolated from marine organisms. These compounds with novel structures and

pharmacological importance (*eg.* prostaglandins) further increased the curiosity of the scientists to explore more marine organisms.[7] MNP have been the continued attraction for drug development and neurophysiological probes and the marine biomedical research has now been the popular subject for many recent reviews.[5d,e,f] These compounds, like tetrodotoxin, shall however command the field of molecular biology.

The main sources of MNP are marine microorganisms and phytoplankton, green algae, brown algae, red algae, sponges, coelenterates, bryozoans, mollusks, tunicates (ascidians), echinoderms along with some minor occurrence in some polychaete worms, fin fishes and crustaceans. The abundance of the source of MNP from the organisms is approximately in the order:

*Sponge* > *Coelenterates* > *Microorganisms & Phytoplankton* > *Echinoderms* = *Tunicates* > *Red algae* > *Mollusks* ≥ *Brown algae* ≥ *Green algae* > *Bryozoans*

Sponges, coelenterates, macro algae and echinoderms are the major contributors. The order of abundance in the type of compounds reported may be given as: (reported for the year 2002)

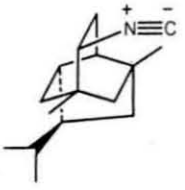
*Terpenoid* > *Polyketide* > *Alkaloid* > *Peptide* > *Shikimate*

Terpenoids are prevalent among sponges and coelenterates.

Reports have been compiled based on the compound types and also on the animals of each phylum. The symbiosis existing between marine organisms in the marine environment was very much correlated to the terrestrial insect chemical ecology by the MNP chemists as this association to every organism was inevitable in terms of the organism's defense, camouflage, *etc.* to protect them from enemies. Initially MNP chemistry was widely developing in three tracks *viz.* marine toxins, marine biomedicines ad marine chemical ecology. Now they have been integrated to give vigour to this field. Due to the interdisciplinary nature of the work, it is difficult to refer any single compendium for the details of MNP research. The identification of the species and phylum during the chemical work is the integral part enabling one to

correlate the result of the work for the significance and status of the particular identified compound with respect to other members of the family and the marine ecosystem. Some of the selective MNP of interest are to be listed in the following sections.

The abundant presence of defensive compounds in the shell-less animal like nudibranch, *Aplysia* spp. is helpful to drive away its predators. One such compound is a sesquiterpene isonitrile, 9-isocyanopupukeanane, (**1**).[8]




**1**

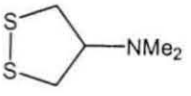
**Micro and macro algae**

Micro algae or diatoms are the primary producers of the food in marine ecosystem. The organic compounds present in them are transformed to other organisms feeding on them, through food web. The compounds of the micro algae are complex in nature and good account of them has been reviewed by Faulkner.[3] Some of the simple and interesting compounds are indicated here. Amphidinolide B, a cytotoxic macrolide has been isolated from dinoflagellate *Amphidinium* spp.[9a] Stypoldione[9b,c] is ichthyotoxic and cytotoxic isolated from brown alga, *Stypopodium zonale*. Zonarone[10] is a fungitoxic hydroquinone obtained from brown seaweed, *Dictyopteris zonaroides*.

Cyclic polysulphides (**2**, **3**) present in the red alga *Chondria californica* have antibiotic activity.[11] One of these compounds having analogous structure called nereistoxin (**4**) isolated from the polychaete worm *Lumbriconereis heteropoda*[12] was used to develop an insecticide called *Padan*.[13]

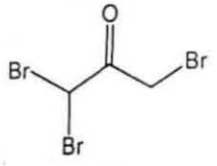

**2**


**3**




**4**

The derivatives of 3-oxoundecyl mercaptan[14] have been isolated from brown algae *Dictyopteris plagiogramma* and *D.australis* and *trans*-7-methoxytetradec-4-enoic acid from *Lyngbya majuscula* and they form the derivatised natural product of higher organisms like gorgonian coral[15] and sea hare[16] respectively. Inseparable polyhalogenated acetones, polyhalogenated butenones and other volatile halogenated compounds (**5**, **6**, **7**) were obtained from red alga *Asparagopsis taxiformis*.[17] The structures were proposed based on the GC-MS data. Fimbroids, a series of halogenated lactones (**8**, **9**, **10**) having antibiotic activity, were isolated from red alga *Delisea fimbriata*.

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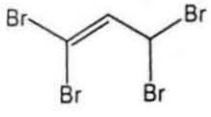


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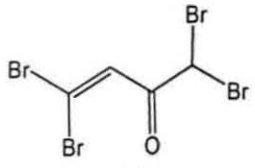
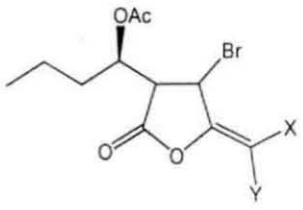



**7**

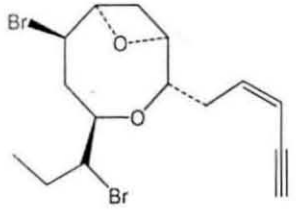
Laureatin (**11**) and isolaureatin (**12**) isolated from red algae *Laurencia* spp. commanded lengthy process of structural elucidation both by PMR and X-ray analysis.[18] The halogenated acetylenic ether, rhodophytin (**13**)[19] is a structurally interesting compound isolated from *L.yamada*. *cis*-Manoenone A (**14**) and isomanoenone A (**15**) were isolated from *L.nidifica* alongwith other isomers.[20] Polyhalogenated monoterpenes first found in sea hares were later confirmed to be present in algae. The structures were also challenging and determined by the combination of both NMR and X- ray studies. Compound (**16**) first found in the sea hare, later found to be originally from red algae *Plocamium cartilagineum*, a principal component of sea hare's diet.[21]




**8** X = H, Y = Br

**9** X = Br, Y = H

**10** X = H, Y = I

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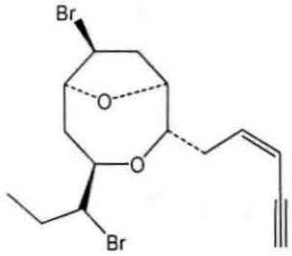


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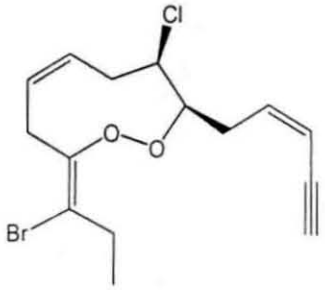


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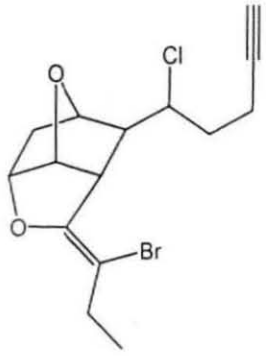


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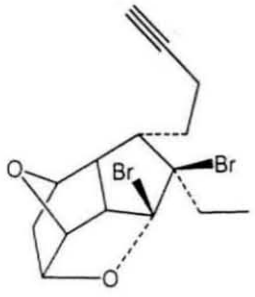
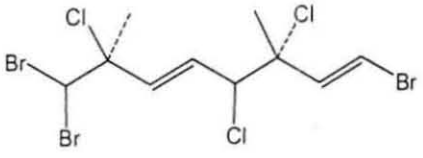



**15**

The red algal species of *Laurencia* is an important source of variety of halogenated sesquiterpenes [*eg.* antibiotic phenolic sesquiterpenes laurinterol (**17**)[22] and its other isomer (**18**)[23]], diterpenes and acetylenes with many carbon skeletons[24] having antibiotic activity.

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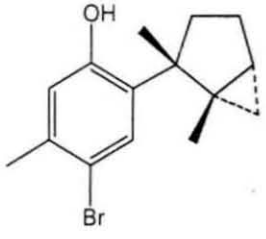


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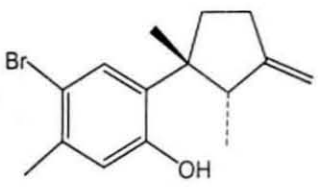


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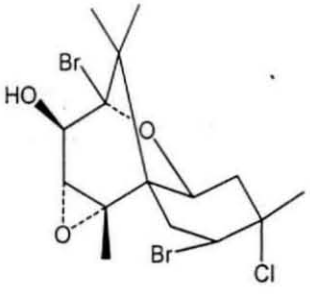
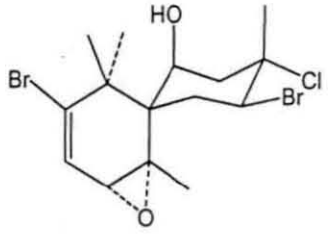



19

Halogenated chamigrene derivatives (**19**, **20**, **21**)[25] are the most frequently reported group of compounds isolated from this genus since the report of pacifenol (**22**) from *L. pacifica*.[26]

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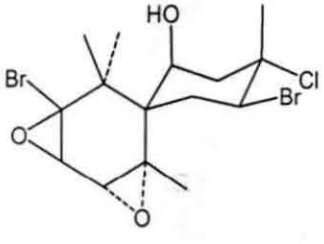


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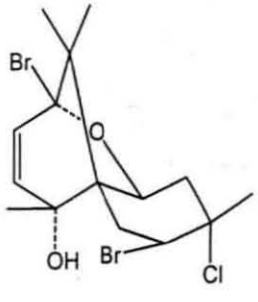
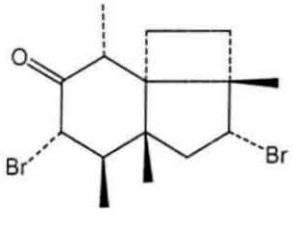
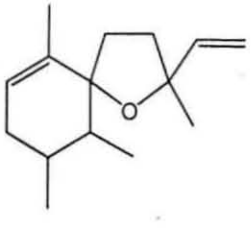



22

Perforatone (**23**) is one of the unusual sesquiterpenes isolated from *L. perforata* among other products.[27] Dactyloxene-B (**24**) is the non-halogenated product obtained from *Laurencia* spp.[28]



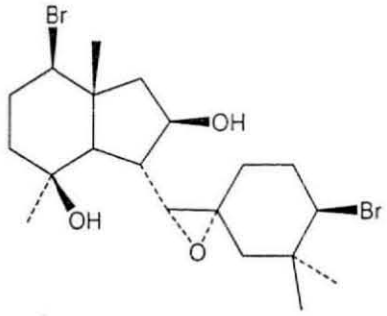

23



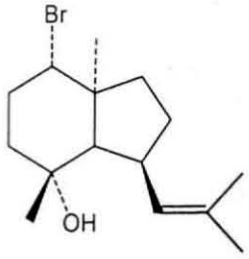

24

Oppositol (**25**)[29] is one of the many metabolites isolated from *Laurencia subopposita* and iriediol (**26**)[30] from a *Laurencia* spp. The striking feature is the

opposite absolute configuration between (**25**) & (**26**) isolated from the same organism group.

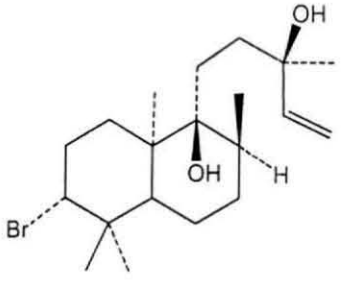



**25**

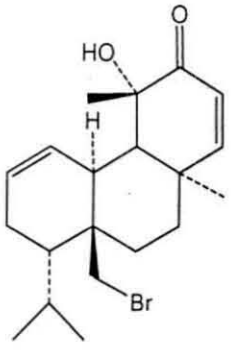



**26**

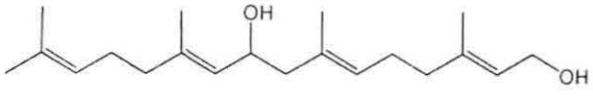
Brominated diterpenes, concinndiol (**27**)[31] and sphaerococcenol A (**28**)[32] were isolated from *Laurencia concinna* and *Sphaerococcus coronopifolius* respectively. Diterpenes are frequently found among brown algae. Crinitol (**29**),[33] a linear diterpene and a monocyclic diterpene (**30**)[34] have been isolated from *Cystoseira crinita* and *Caulerpa brownii* respectively.



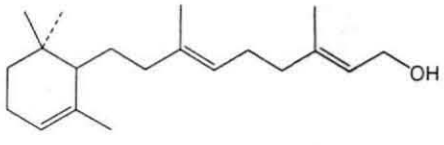

**27**




**28**

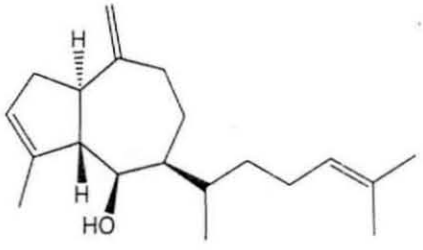



**29**




**30**

Pachydictyol A (**31**)[35] has been found in *Pachydictyon coriaceum* and its structural analogue, dictyol A (**32**)[36] is found in *Dictyota dichotoma*.

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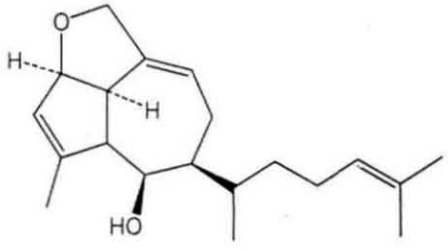
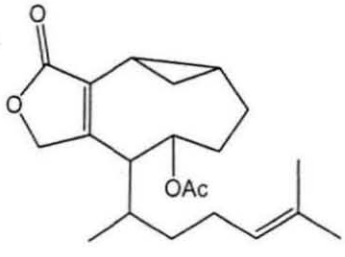
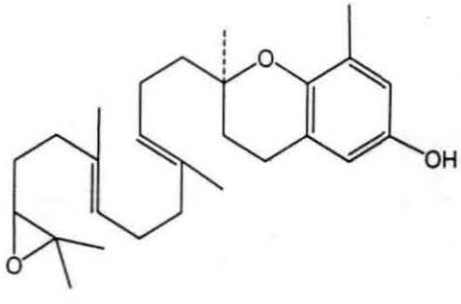



32

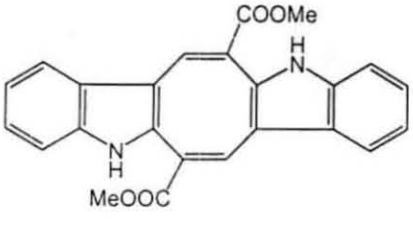
Acetoxycrenulatin (**33**)[37] isolated from *D. crenulata* has quite a different carbon skeleton. The epoxide of $\delta$-tocotrienol (**34**)[38] from a brown algae *Sargassum tortile* was found to induce settling of a hydroid on it. Caulerpin (**35**),[39] a pigment isolated from green algae of genus *Caulerpa* was found to contain two units of tryptophan. Brominated phenols like lanosol (**36**) are found in many red algal varieties.



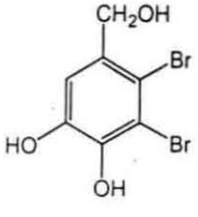

33




34

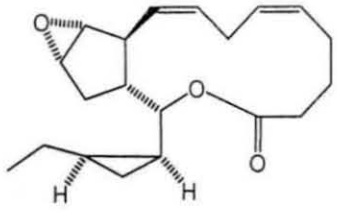



35

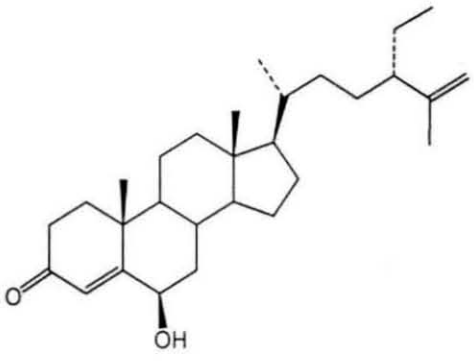



36

Prostanoid analogues have also been reported from the red algae *Gracilaria edulis*[40] and *Laurencia hybrida*. [eg. Hybridalactone (**37**)].[41] A series of oxygenated steroids like (**38**)[42] have been isolated from green algae *Codium arabicum*.




**37**



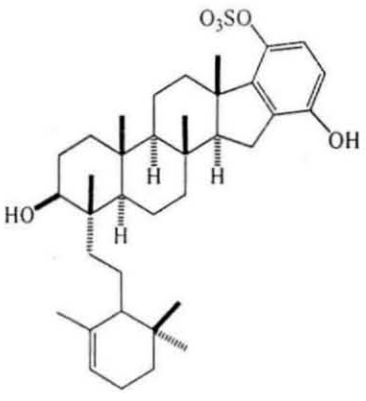

**38**

**Sponges**

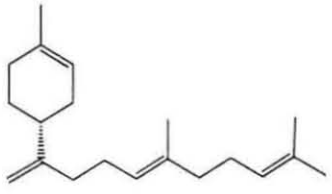
Sponges are one of the major contributors of novel MNP. More reports continue to pour in regarding this organism. As this is the storehouse of various marine biota, symbiotic association of each organism depends on one another for their mutual benefits. Many of the sponge compounds now have become the precursor for the development of new drugs. Some of the selected compounds with potential biological activity are listed here.

**Adociasulfate 5 (39)** - *Adocia* sp.,[43] hexaprenoid hydroquinones, cytotoxic, inhibitor of motor protein kinesin.

**Axinyssene (40)** - *Axinyssa* sp.,[44] mild cytotoxic diterpene

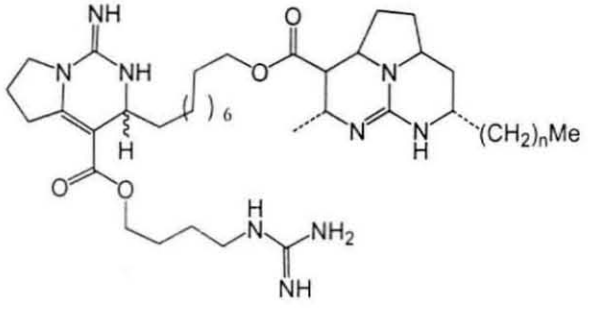



39



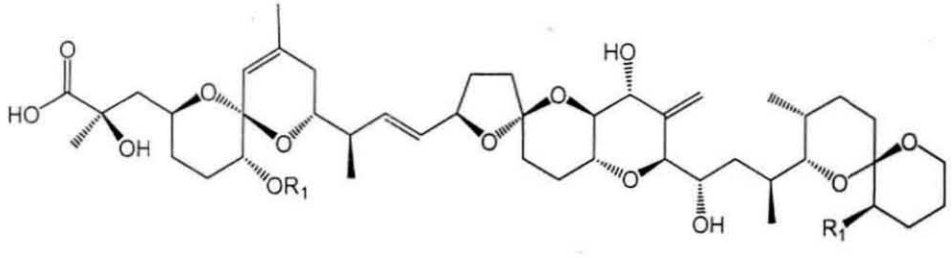
40

**Batzelladines– Batzelladine A (41)**, *Batzella* sp.,[45] inhibitor of AIDS




41

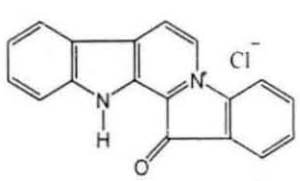
**Okadaic acid (42)** – *Halichondria okadai*,[46] protein phosphatase inhibitor



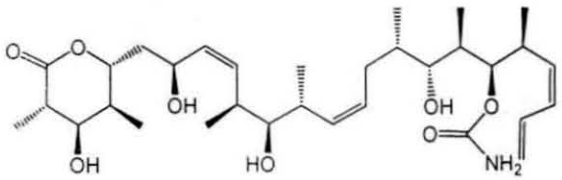

**42**
**$R_1$ = H, $R_2$ = H/$CH_3$**

**Fascaplysin (43)** - *Facaplysinopsis* sp.,[47] antimicrobial and cytotoxic

**Discodermolide (44)** - *Discodermia dissoluta*,[48] cytotoxic and immunosuppressive agent



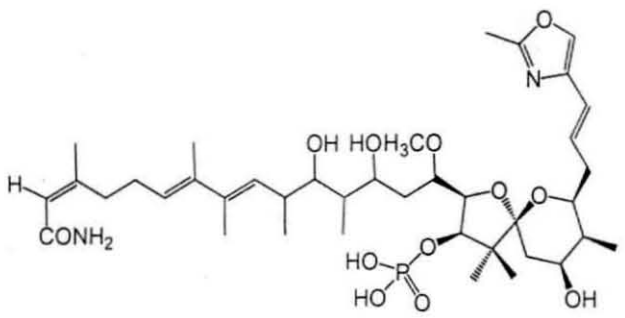
43



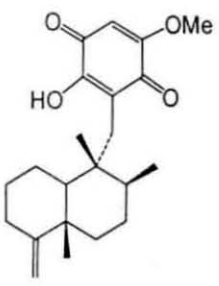
44

**Geometricin A (45)** - *Luffariella* sp.,[49] calyculinamide derivative, moderate cytotoxicity, anti-algal

**Ilimaquinone (46)**, - *Dactylospongia* sp.,[50] sesquiterpene, low antimicrobial and low cytotoxicity, causes vesiculation of the Golgi



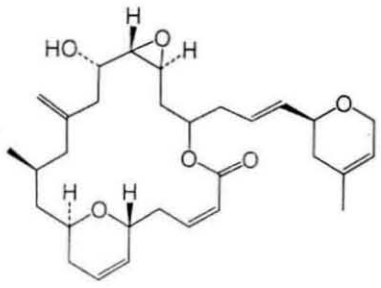
45



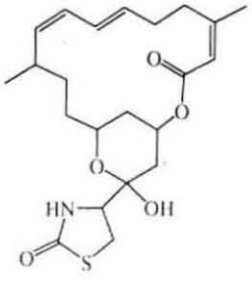
46

**Fijianolide-B (47)** - *Spongia mycofijiensis*,[51] Polyketide heterocycles, cytotoxic macrocyclic lactones, antitumour and anthelminthic

**Latrunculin A (48)** - *Latrunculia magnifica*,[52] ichthyotoxic, cytotoxic



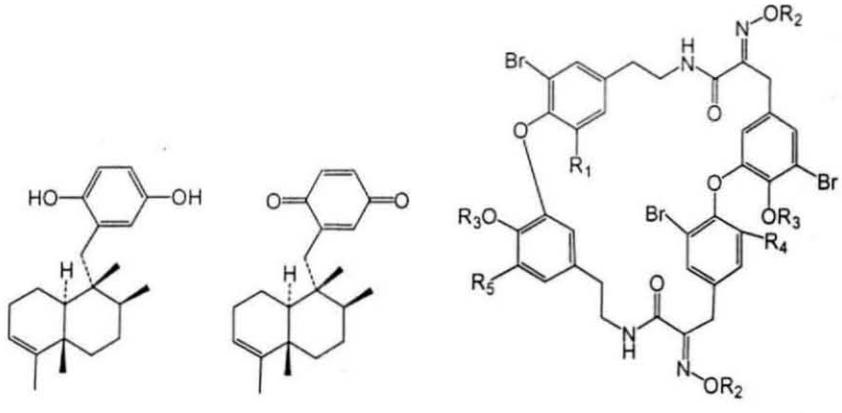
47



48

**Averol** (**49**) and **Avarone** (**50**) – *Dysidea avara*,[53] anti HIV

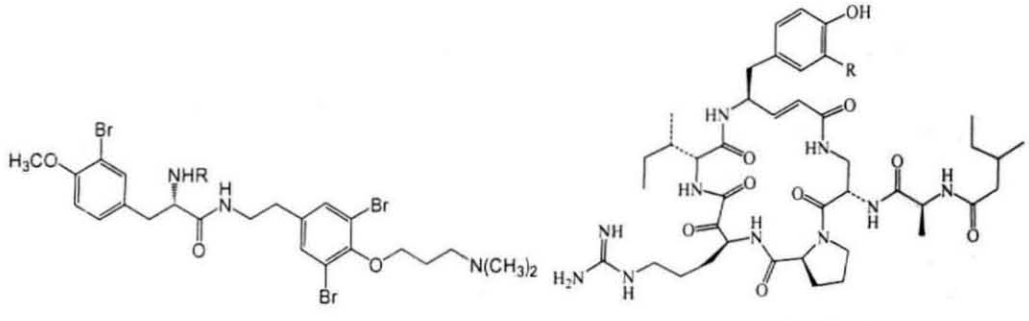
**Bastadins** (**51**) - *Ianthella basta*,[54] highly modified tetrapeptides and macrocyclic, antimicrobial, *in vitro* cytotoxic activity against human tumor cell lines P388 or anti-inflammatory activity




**49** **50** **51**

**Suberedamine A** & **B** (**52**, **53**) - *Suberea* sp.,[55] cytotoxic bromotyrosine alkaloids

**Cyclotheonamide E4** & **E5** (**54**, **55**) - *Ircinia* sp.,[56] inhibit cell growth

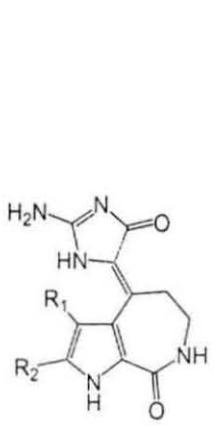



**52**, **R** = **H**
**53**, **R** = **Me**

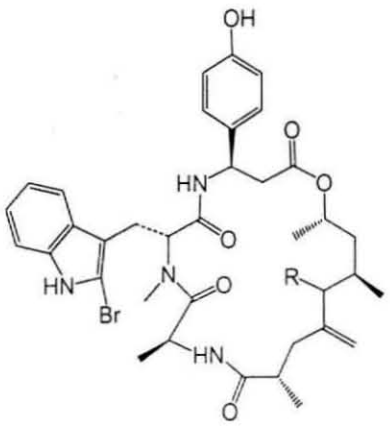
**54**, **R** = **H**
**55**, **R** = **OH**

**Debromohymenialdisine** (**56**) & **Hymenialdisin** (**57**) - *Hymeniacidon* sp.,[57] bromopyrrole alkaloids called spongiacidins, anti-inflammatory agent

**Jaspamide** (=**Jasplakinolide**) (**58**) - *Jaspis* sp.,[58] cyclodepsipeptide, reagent in cell biology to act on actin, insecticidal and antifungal



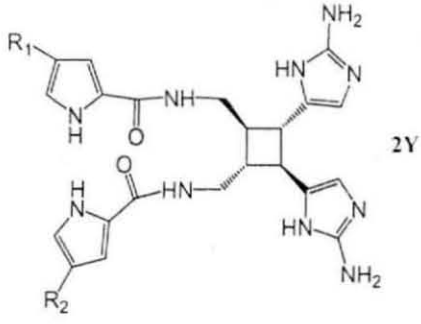
56, $R_1$&$R_2$ = H
57, $R_1$ = H, $R_2$ = Br



58

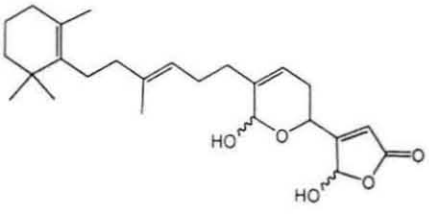
**Sceptrins (59)** – *Agelas sceptrum*,[59] antimicrobial

**Manoalide (60)** - *Luffarriella variabilis*,[60] standard drug for irreversible inhibitor of phospholipase $A_2$, potent anti-inflammatory activity and pain killing agent



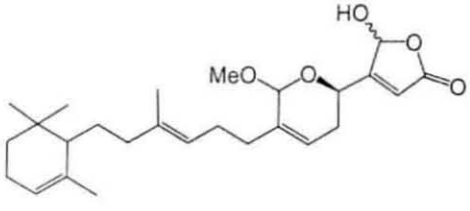
59, $R_1$ & $R_2$=H/Br
Y=HCl/AcOH

2Y

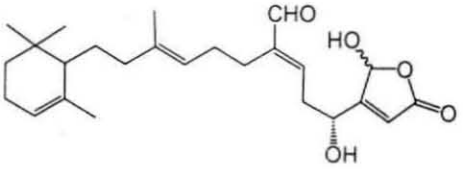


60

**Luffariolide H & J (61, 62)** - *Luffariella* sp.,[61] sesterterpene, mild cytotoxic



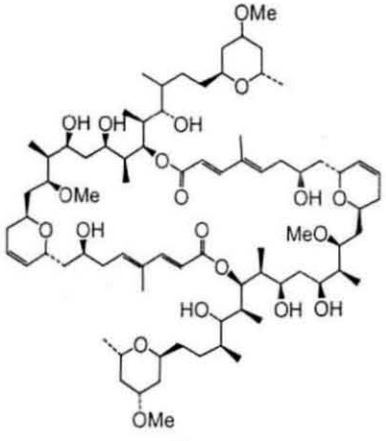
61



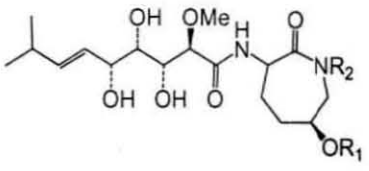
62

**Swinholide A (63)** – *Theonella swinhoei*,[62] reagent in cell biology to act on actin

**Bengamides (64)** – *Jaspis carteri*,[63] specific pharmacological activities with differential cytotoxicity against human tumour cell lines



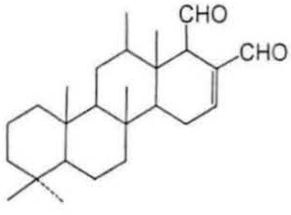

63



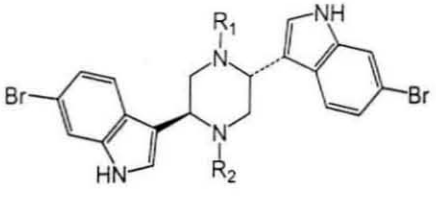

64, $R_1$,$R_2$ = H/Me

**Scalaradial (65)** – *Cacospongia mollior* sp., *Spongia officinalis*,[64] tetracarbocyclic sesterterpene

**Dragmacidins (66)** - *Spongosorites* sp., *Dragmacidon* sp.,[65] enzyme inhibitor, antiviral



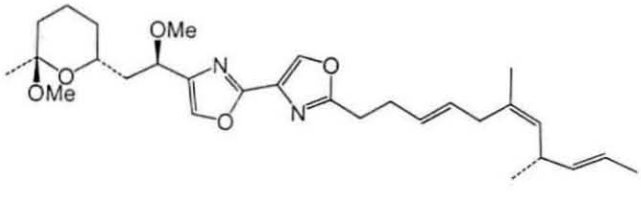

65



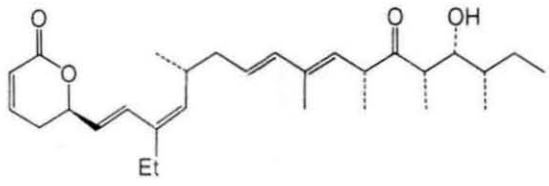

66

**Hennoxazole A (67)** – *Polyfibrospongia* sp.,[66] antimalarial and antiviral

**Callystatin A (68)** – *Callyspongia truncata*,[67] polyketide, high cytotoxicity




67




**68**

As the explorations of MNP is of proliferating nature, focus on biological properties has also been emphasized with about 50% of the compounds reported in 2003 have undergone these tests. Testing for anticancer and antimicrobial properties dominate among the five categories of testing. Sponges are one of the sources of detection of potential anti-cancer compounds. Sponges and coelenterates continue to dominate as source phyla of new compounds. The inheritance of the latest drugs is obviously has the origin with these explorations of marine fauna and flora as has been seen in the case of some of the antiviral drug like acyclovir and AZT.[1] Few others to quote are *Yondelis* better known as ecteinascidin 743 and conus toxin *Ziconotide* or *Prialt* in Phase II/III trials against soft tissue sarcoma and Phase III trials for intractable pain respectively.[68]

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Chapter 2

# Experimental procedures

**General Remarks on Experimental Procedures**

- All solvents were freshly distilled before use. Evaporation of the solvents was done on Büchi rotary vacuum evaporator below 40 °C under reduced pressure.
- Column chromatography was performed on silica gel 60-120 mesh (s.d. Fine Chemicals) by wet pack method and eluted with petroleum ether 60-80°C, ethyl acetate, and methanol.
- Sample was dissolved in solvent and spread on little silica gel and dried under air before loading the column.
- *tlc* was performed on small glass strips prepared from silica gel G (Merck) dissolved in water. The spots were located by blowing iodine vapour on the developed plates.
- IR spectra were recorded on Jasco FT-IR instrument
- $^{1}H$ NMR, $^{13}C$ NMR and 2D NMR of the samples were recorded on Bruker 300 MHz instrument dissolved in $CDCl_3/D_2O$, with tetramethylsilane (TMS) as internal standard.
- The GC-MS analysis of the samples were done using Finnigen *Trace* GC and *Trace* MS. The oven temperature was set up at 250°C. The sample dissolved in $CHCl_3$ (~0.6 to 0.9 %, 1.2 µl) was injected. Nitrogen was used as carrier gas. The mass spectral library software "**NIST**" was used as the standard reference for comparison with that of the sample compound peak.
- An LG domestic microwave oven (one cubic litre capacity) has been used for microwave assisted reactions

**Sponges and finfish collection and identification**

**Location of study area**

In order to explore the presence of possible bioactive compounds from the marine organisms of the South East Indian coast, the shallow water sponges of Mandapam coast were collected for the present study. The Gulf of Mannar (GoM) and Palk Bay (PB) are rich in marine flora and fauna. Mandapam peninsula consists of PB

in the north and GoM in the south. The GoM lies between 8°46' and 9°14' N latitude and 78°9' and 79°14' E longitude between Mandapam town in the North to Tuticorin in the South with a distance of 170 nautical miles. PB coast from Pamban and Rameswaram Island at 9°17' N and 79°15' E was used as the source for collection of sponge specimens. So far, 4562 different species of sponges have been identified in the world. Among them 486 species of sponges have been noticed in India[1] and 275 species have been found in GoM-PB.[2] All sponge specimens were collected live, frozen and stored in methanol until start of extraction work.

**Collection and identification**

Three specimens *viz. Siphonochalina* spp., *Hippospongia* spp. and *Spongia* spp. were collected from PB of Mandapam coast and two specimens viz. *Cervicornia* spp. and *Hyrtios* spp. from Rameswaram Olaikuda coast during May 2003. The genera of the specimens were identified by Dr. Anita Mary, Dept of Coastal area studies, Manonmaniam Sundaranar University, Marina Campus, Rajakkamangalam, Kanyakumari District, Tamil Nadu, India. The classification followed in Hooper's 'Sponguide' has been used for the identified sponges.[3] The live sponges after collection were stored in plastic containers containing methanol after freezing them overnight. They were diced before packing and individually analyzed in the laboratory. All the sponges were subjected to the preliminary study involving simple experimental techniques of *tlc* and silica gel column chromatography to get moderate pure fractions from the crude extract residues. The semi pure fractions were then analyzed by gas chromatography-mass spectrometry (GC-MS). The puffer fishes, *Tetraodon* spp. were procured in fresh dead condition caught from the Palk Bay during June 2002. The fishes were immediately frozen before subjected to extraction. The identity of the fish was compared with the standards available in the book for fish identification, in the museum of CMFRI, Mandapam Camp, Ramanathapuram District, Tamil Nadu.

**Determination of antifungal characteristics of the synthesized compounds**

The assay was made by poison food technique, where the desired quantities of the synthesized compounds were incorporated in molten Potato Dextrose Agar medium. A 2 mm agar block of five days old culture of test fungi was placed on the center of the petri plate containing PDA medium amended with appropriate concentration of synthesised compounds. The plates were incubated at 25 °C ±2 for 7 days. The inhibitory ranges and sensitiveness were determined by recording the mycelial growth after the incubation period. All the experiments have been carried out in triplicates.

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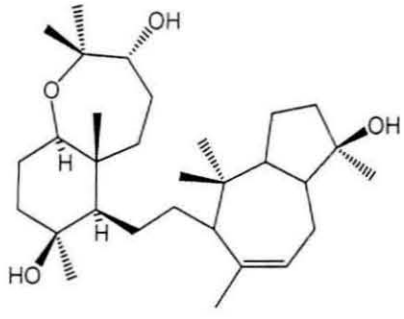
Chapter 3

# Chemical investigation of the sponge *Siphonochalina* spp.

**Compounds of sponge *Siphonochalina* spp.**

The sponge *Siphonochalina* spp. was found to be a rich source of triterpenoids among other compounds reported so far. These triterpenes were squalene derived compounds with three different skeleta, namely, sipholanes, siphonellane and neviotane as have been reported by Kashman *et al.*[1-5] Among the terpenoids isolated so far, triterpene is a rare occurrence among sponges, though mokupalide has been isolated by Scheuer *et al.*[6] The squalene derived triterpenes *viz.* sipholenol-A (**1**) and sipholenone-A (**4**)[1] with new ring system of sipholane were isolated from *Siphonochalina siphonella* (Levi, 1965).

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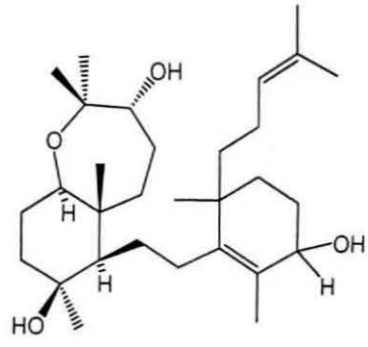
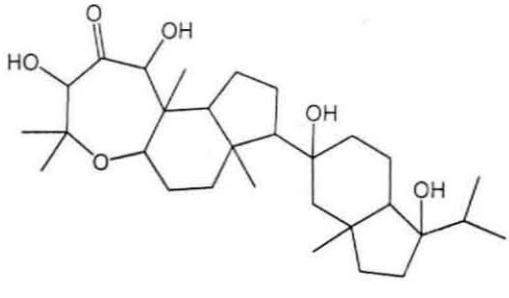



2

Further investigation of *S. siphonella* led to isolation of ten additional sipholanes as minor components.[2] Siphonellinol-A (**2**)[3] is having siphonellane base unit.

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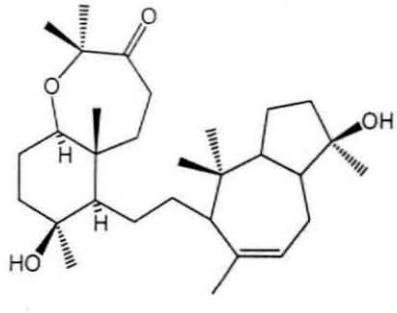
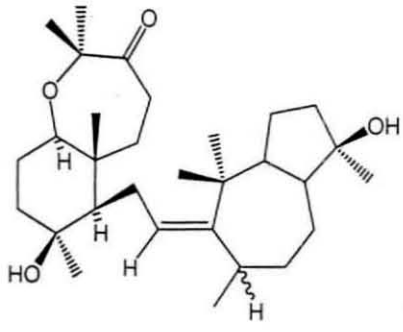



4

Neviotine-A (**3**)[4] isolated later was a pentacyclic compound related to the previously isolated sipholanes and siphonellanes with neviotane skeleton. All the three groups have common perhydrobenzoxipine moiety. Recently nine new compounds were isolated

from Red Sea sponge, *S. siphonella*.[5,7] They are sipholenone-D (**5**), sipholenol-F (**6**), sipholenol-G (**7**), sipholenol-H (**8**), sipholenoside-A (**9**), sipholenoside-B (**10**), siphonellinol-B (**11**), neviotine-B (**12**) and dahabinone-A (**13**).



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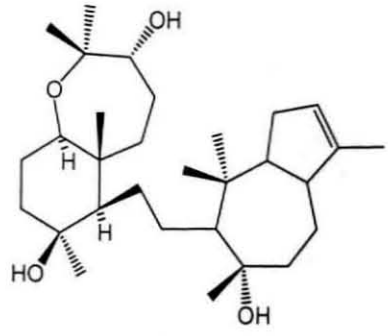

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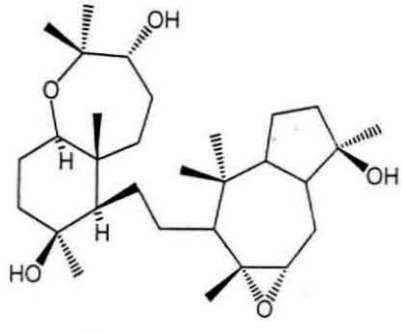

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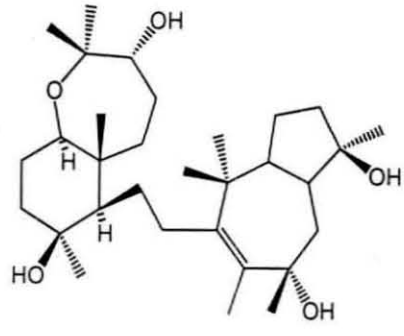

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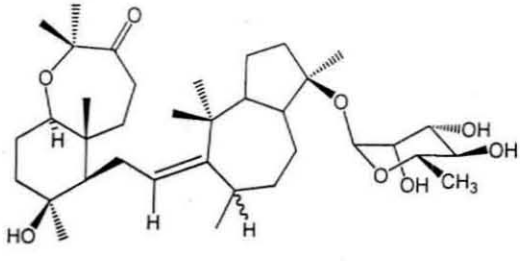

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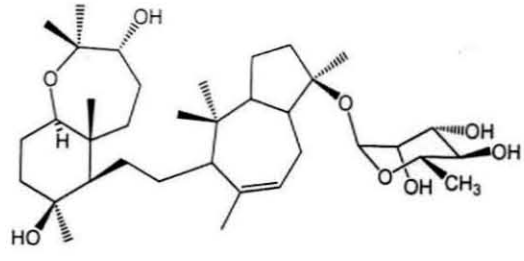

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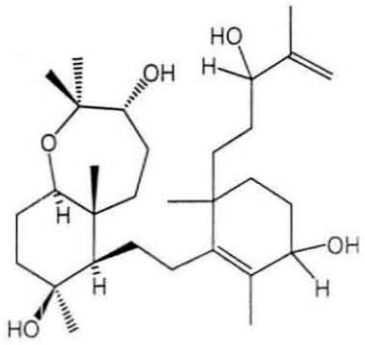


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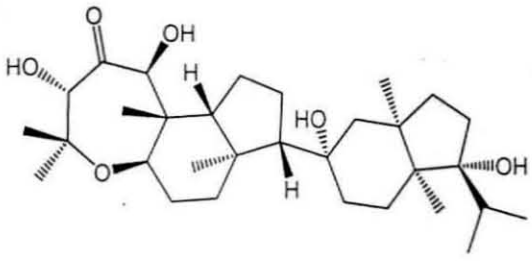


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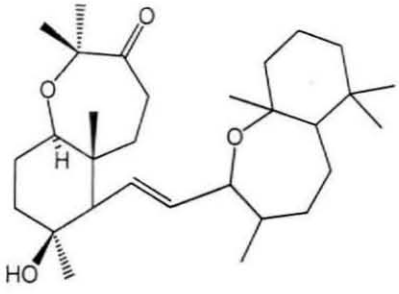
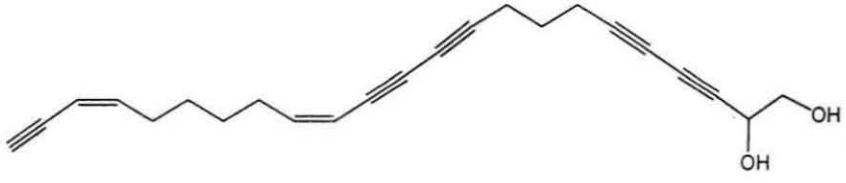



13

$C_{23}$ Polyacetylenic diols like siphonodiol[8] (**14**), dihydrosiphonodiol (**15**) and tetrahydrosiphonodiol (**16**) which were found to be H, K-ATPase inhibitors, were isolated from another species, *S. truncata*.[9]

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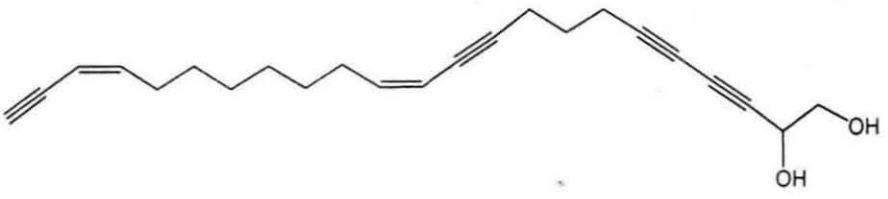



16

Likewise chemical and biological investigations of extracts of a *Siphonochalina* sp. have revealed the presence of potent antitumour hemiasterlin class of compound, hemiasterlin-C. It was found that the hemiasterlins isolated were more potent than dolastatin-15, equipotent with cryptophycin-1 and somewhat less potent than dolastatin-10, during cytotoxic and antitubulin activity experiments.[10]

This being the importance of bioactive principles from *Siphonochalina*, it was decided to investigate the chemical composition of this sponge available in GoM-PB and the results are presented here.

**Chemical Analysis of Sponge *Siphonochalina* sp.**

The specimen (**Fig -1**) weighed 875 g when collected and was stored in 650 ml of methanol. The classification of this sponge is given as:

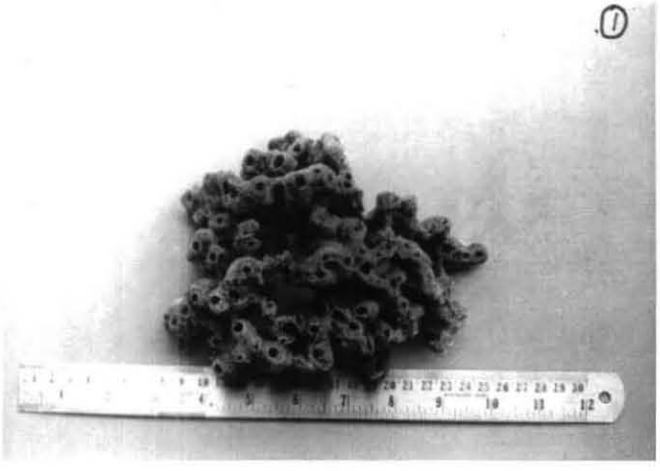
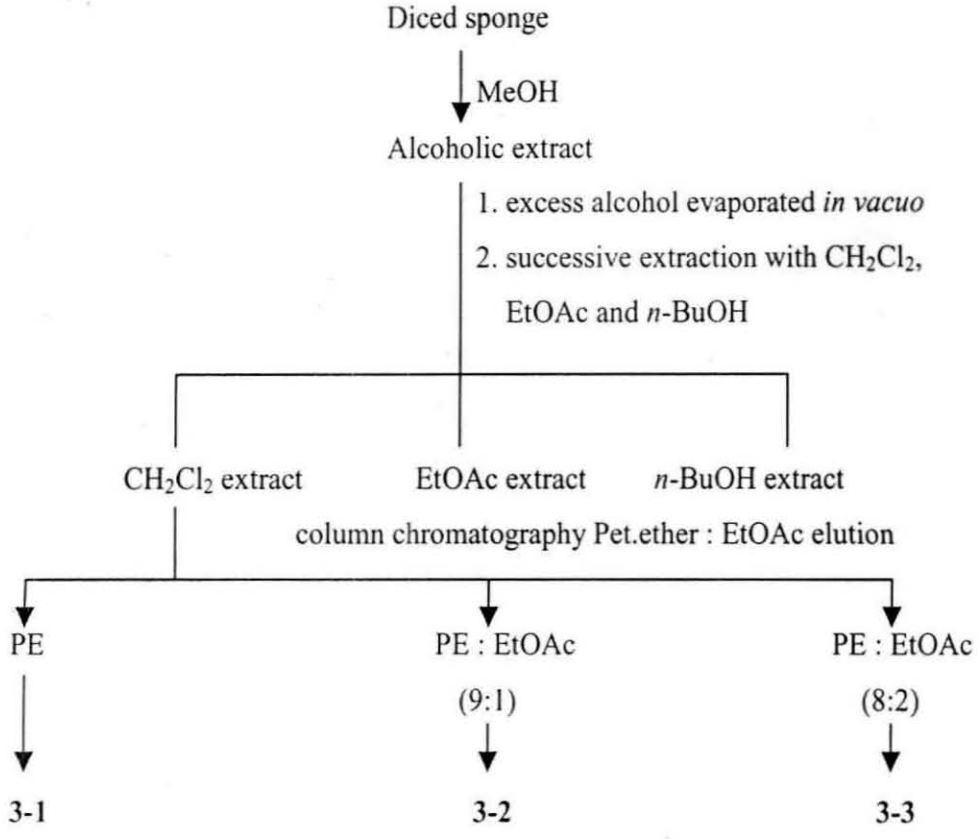


**Fig – 1**

| | | |
|---|---|---|
| Phylum | - | Porifera |
| Class | - | Demospongia |
| Sub class | - | Ceractinomorpha |
| Order | - | Haplosclerida |
| Family | - | Callyspongiidae(de Laubenfels 1936) |
| Genus | - | *Siphonochalina* spp.(Schmidt 1868) |

Literature review showed no report on chemical investigation on this species of GoM-PB area. So it was felt appropriate to investigate this specimen chemically. The extraction and fractionation of crude extract are depicted in **Scheme - 1**

**Extraction and Fractionation of *Siphonochalina* sp.**




**Scheme - 1**

The sponge stored in methanol was squeezed and further extracted with 1300 ml of fresh methanol. The extracts were combined, filtered and excess alcohol was evaporated *in vacuo* below 40 °C. The concentrated crude alcoholic extract was then air evaporated and successively extracted with dichloromethane, ethyl acetate and *n*-butanol. Each extract was evaporated to recover the corresponding residue. The residues were checked by *tlc* (EtOAc:EtOH – 2:1) before purifying them by column chromatography. Residue of dichloromethane extract was column chromatographed over silica gel (Merck, 60-120 mesh) and eluted with petroleum ether with increasing concentration of ethyl acetate while monitoring each lot of eluates by *tlc* (Pet. ether, EtOAc-3:1) to get three semi pure fractions *viz.* **3-1** to **3-3,** all of them being reddish yellow residue.

Similar column chromatography of ethyl acetate and *n*-butanol extract residues did not yield any pure component. The aqueous alcoholic layer left after these extractions was air evaporated and checked by *tlc*. It gave only streaks in different solvent systems.

Analysis of fraction **3-1 (Table - 1)**

This fraction shows varying compositions of both saturated and unsaturated long chain fatty acid methyl esters in GC-MS. The spectral details of nine compounds (**3-1-1** to **3-1-9**) are shown in **Table - 1**. The chain length varies from $C_{13}$ to $C_{18}$. The compounds showed $M^+/M+1$ peaks. The structures of compounds have all been confirmed by comparing with library.

The base peak at *m/e* 74 is observed in all saturated straight chain methyl esters **3-1-1**, **3-1-3**, **3-1-4**, **3-1-7** and **3-1-9** arising out of McLafferty rearrangement. Unsaturated esters **3-1-5** (**Fig - 3**), **3-1-6** (**Fig - 4**) and **3-1-8** (**Fig - 5**) give rise to *m/e* 55 for $[C_4H_7]^{+\cdot}$ unit along with *m/e* 69, 83, 97, 111... with mass unit of 14 as difference with higher value of fragment ion depending on the chain length. This pattern is typical for the unsaturated fatty esters. The cleavage of $C_3$-$C_4$ bond gives a peak at *m/e* 87, which is dominant in all the compounds. The compound **3-1-2** (**Fig - 2**) shows the base peak at *m/e* 87 due to the formation of $[CH_2CH_2COOCH_3]^{+\cdot}$ unit. Compound **3-1-6** has been reported to be isolated from a sponge and synthesized.[11] It is also present in whale blubber oil[12] and sea buck thorn pomace.[13]

**Table - 1**: Analysis of fraction **3-1**

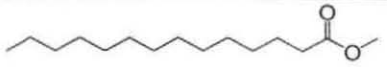
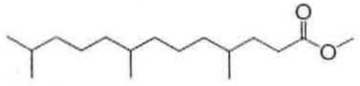
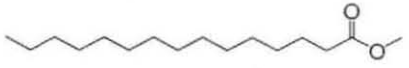
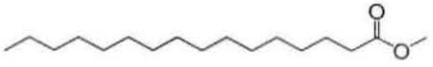
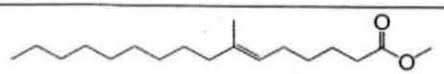
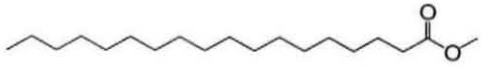
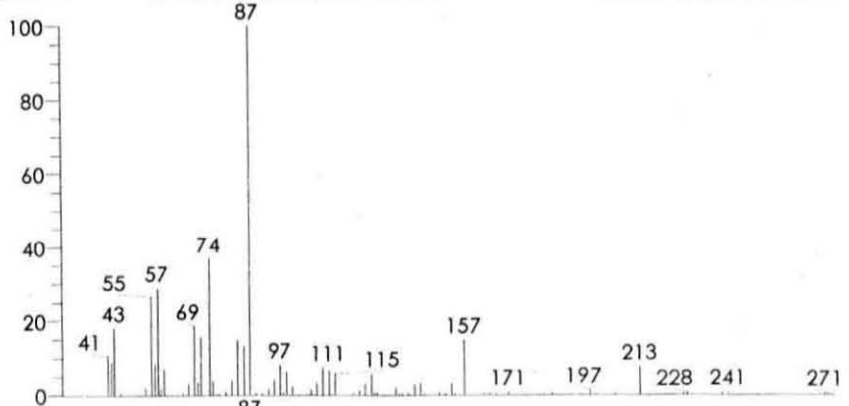
| Compd No. | RT | Peak area %age | MF | MW | Structure | 1st MS Peak | Prominent peaks in m/e (1st Peak = Base Peak) |
|---|---|---|---|---|---|---|---|
| **3-1-1** | 8.72 | 4.28 | $C_{15}H_{30}O_2$ | 242 |  Methyl tetradecanoate | 242 | 74, 87, 43, 57 |
| **3-1-2** | 9.32 | 26.89 | $C_{17}H_{34}O_2$ | 270 |  Methyl 4,8, 12-trimethyl tridecanoate | 271 | 87, 74, 57, 213 |
| **3-1-3** | 9.45 | 14.06 | $C_{16}H_{32}O_2$ | 256 |  Methyl pentadecanoate | 256 | 74, 87, 57, 43, 143, 213 |
| **3-1-4** | 10.46 | 4.52 | $C_{17}H_{34}O_2$ | 270 |  methyl hexadecanoate | 271 | 74, 87, 42, 55, 143, 227 |
| **3-1-5** | 10.68 | 17.67 | $C_{17}H_{32}O_2$ | 268 |  Methyl hexadecenoate | 269 | 55, 74, 87, 69, 96, 43, 152, 194, 237 |

**Table - 1 Contd...**

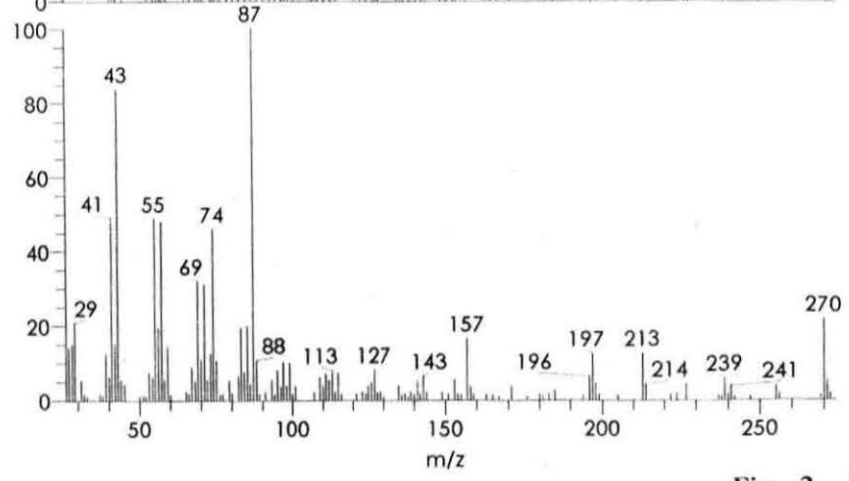
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **3-1-6** | 11.40 | 2.50 | $C_{18}H_{34}O_2$ | 282 |  Methyl 7-methyl 6-hexadecenoate | 242 | 74, 87, 43, 57 |
| **3-1-7** | 11.44 | ~1.20 | $C_{18}H_{36}O_2$ | 284 |  Methyl heptadecanoate | 285 | 74, 42, 87, 55, 143, 241 |
| **3-1-8** | 12.55 | 3.12 | $C_{19}H_{36}O_2$ | 296 |  Methyl 9(Z)-octadecenoate | 296 | 55, 42, 74, 83, 96, 110, 264, 222 |
| **3-1-9** | 12.79 | 2.66 | $C_{19}H_{38}O_2$ | 298 |  Methyl octadecanoate | 299 | 74, 87, 42, 55, 143, 97, 255 |

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 779 | 786 | Tridecanoic acid, · | |
| 2 | 726 | 753 | Tridecanoic acid, · | |
| 3 | 648 | 718 | Methyl 4,8-dimeth· | |
| 4 | 630 | 636 | Tridecanoic acid, | |
| 5 | 613 | 671 | Eicosanoic acid, m | |
| 6 | 610 | 636 | Pentadecanoic ac | |
| 7 | 598 | 599 | Tetradecanoic ac | |
| 8 | 594 | 628 | Dodecanoic acid, | |
| 9 | 593 | 659 | Triacontanoic acic | |
| 10 | 591 | 724 | Undecanoic acid, | |
| 11 | 588 | 685 | Pentadecanoic ac | |
| 12 | 585 | 695 | Undecanoic acid, | |
| 13 | 583 | 649 | Octanoic acid, 4,8 | |
| 14 | 574 | 635 | Undecanoic acid, | |

Tridecanoic acid, 4,8,12-trimethyl-, methyl ester
Formula C17H34O2, MW 270, CAS# 10339-74-9, Entry# 9032
Methyl 4,8,12-trimethyltridecanoate




A-1-2#1023 RT: 9.32 AV: 1
NL: 4.18E6 T: {0,0} + c EI
det=350.00 Full ms [
40.00-600.00]




SI 630, RSI 636, REPLIB, Entry# 9032, CAS# 10339-74-9, Tridecanoic acid, 4,8,12-trimethyl-, methyl ester

Raw data - Library entry

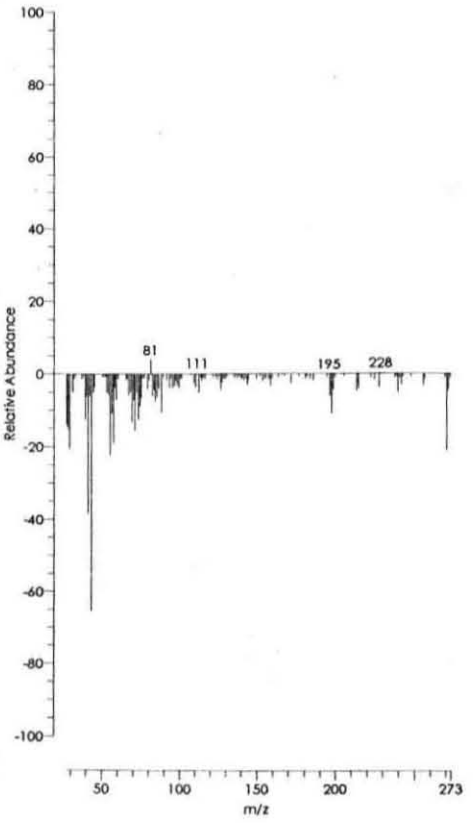
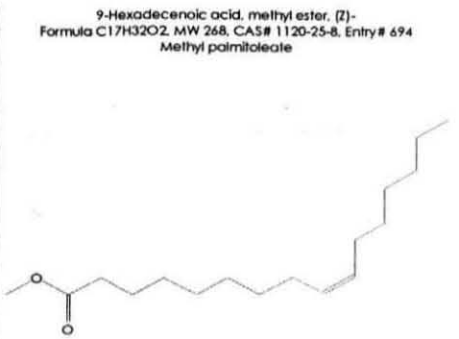
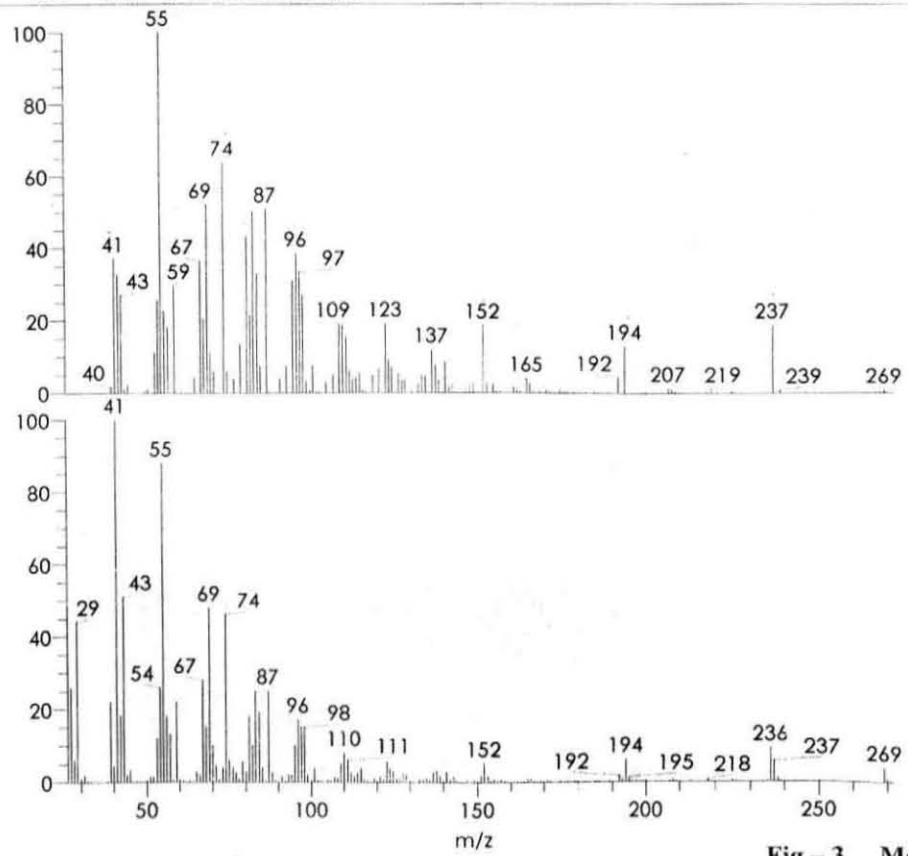



**Fig – 2 Mass spectrum of 3-1-2**

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 817 | 817 | 9-Hexadecenoic a | |
| 2 | 813 | 849 | 9-Hexadecenoic a | |
| 3 | 806 | 807 | 9-Hexadecenoic a | |
| 4 | 802 | 831 | 9-Hexadecenoic a | |
| 5 | 789 | 790 | 9-Hexadecenoic a | |
| 6 | 783 | 784 | 7-Hexadecenoic a | |
| 7 | 773 | 810 | 9-Octadecenoic a | |
| 8 | 765 | 769 | 9-Octadecenoic a | |
| 9 | 765 | 769 | 9-Octadecenoic a | |
| 10 | 723 | 727 | 11-Hexadecenoic a | |
| 11 | 717 | 718 | 6-Octadecenoic a | |
| 12 | 710 | 716 | 2-Hexadecenoic a | |
| 13 | 706 | 755 | 10-Undecenoic ac | |
| 14 | 706 | 706 | 9-Octadecenoic a | |




A-1-2#1181 RT: 10.68
AV: 1 NL: 1.13E6 T: {0,0} + c EI det=350.00 Full ms [ 40.00-600.00]

SI 789, RSI 790, REPLIB, Entry# 694, CAS# 1120-25-8, 9-Hexadecenoic acid, methyl ester, (Z)-

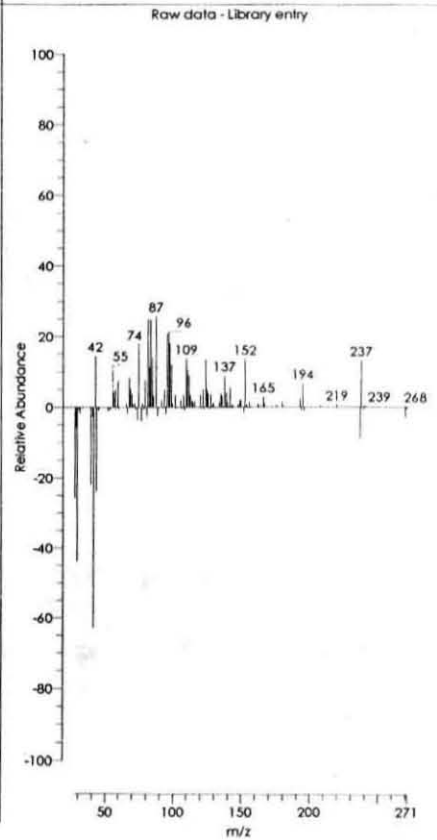
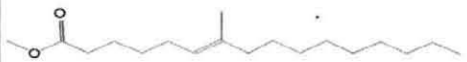
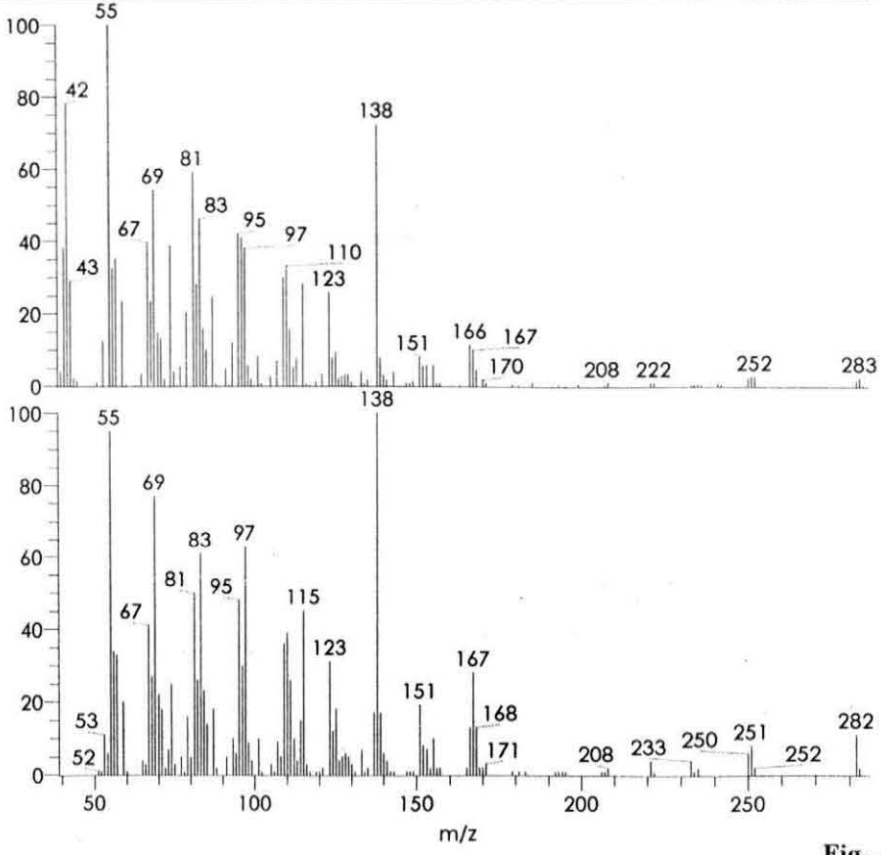



**Fig – 3 Mass spectrum of 3-1-5**

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 775 | 779 | 6-Hexc | |
| 2 | 773 | 776 | 6-Hexa | |
| 3 | 677 | 684 | Oleyl A | |
| 4 | 666 | 685 | 6-Octe | |
| 5 | 659 | 800 | Oleyl A | |
| 6 | 659 | 800 | Oleyl A | |
| 7 | 657 | 667 | 9,12-Oc | |
| 8 | 641 | 679 | 1,12-Dc | |
| 9 | 636 | 702 | 13-Doc | |
| 10 | 630 | 739 | cis-9-Te | |
| 11 | 625 | 625 | Ethano | |
| 12 | 624 | 628 | Z-14-Oc | |
| 13 | 619 | 625 | E-11,13 | |
| 14 | 618 | 638 | Aspido | |

6-Hexadecenoic acid, 7-methyl,methyl ester (E)
Formula C18H34O2, MW 282, CAS# NA, Entry# 62654






A-1-2#1265 RT: 11.40 AV: 1 NL: 3.27E5 T: {0,0} + c EI det=350.00 Full ms [ 40.00-600.00]

SI 775, RSI 779, MAINLIB, Entry# 62654, CAS# NA, 6-Hexadecenoic acid, 7-methyl,methyl ester (E)

Raw data - Library entry

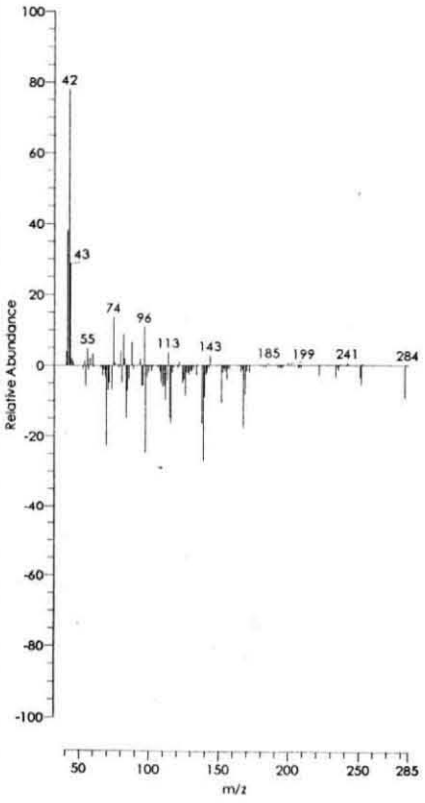
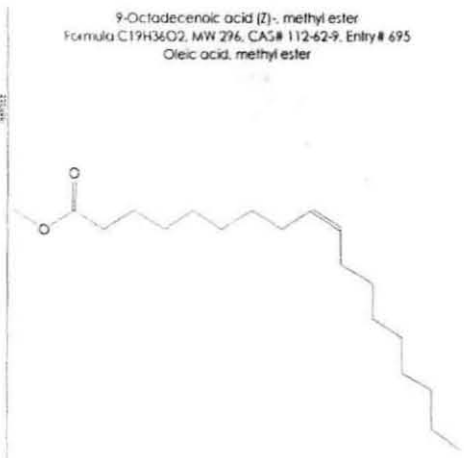
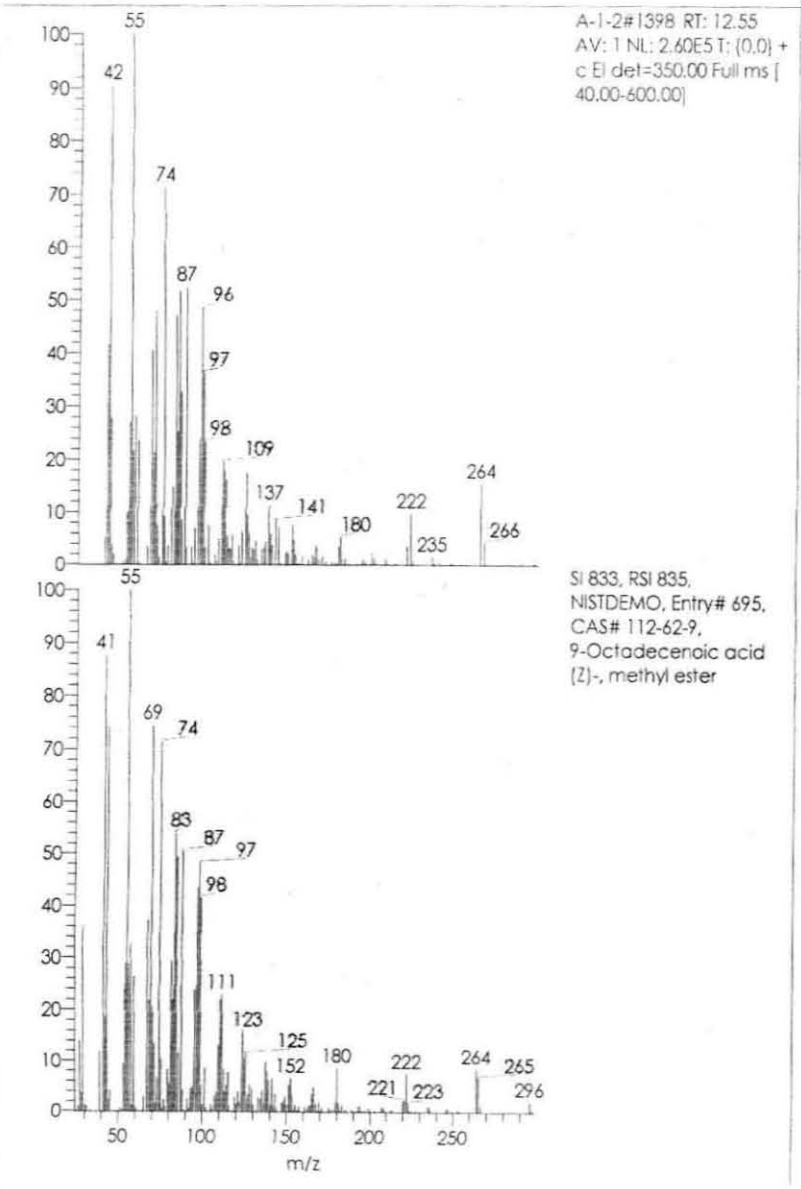



**Fig – 4 Mass spectrum of 3-1-6**

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 852 | 855 | Triolein | |
| 2 | 840 | 846 | 9-Octadecenoic acid (Z | |
| 3 | 833 | 835 | 9-Octadecenoic acid (Z | |
| 4 | 833 | 835 | 9-Octadecenoic acid (Z | |
| 5 | 814 | 815 | 9-Octadecenoic acid (Z | |
| 6 | 811 | 816 | 9-Octadecenoic acid (Z | |
| 7 | 804 | 804 | 9-Octadecenoic acid, n | |
| 8 | 799 | 810 | 9-Octadecenoic acid (Z | |
| 9 | 796 | 796 | 6-Octadecenoic acid, n | |
| 10 | 793 | 793 | 11-Octadecenoic acid, | |
| 11 | 792 | 792 | 9-Octadecenoic acid, n | |
| 12 | 785 | 785 | 6-Octadecenoic acid, n | |
| 13 | 765 | 765 | 8-Octadecenoic acid, n | |
| 14 | 765 | 765 | 7-Octadecenoic acid, n | |
| 15 | 763 | 763 | 10-Octadecenoic acid, | |
| 16 | 760 | 761 | 6-Octadecenoic acid, n | |
| 17 | 759 | 759 | 5-Octadecenoic acid, n | |
| 18 | 758 | 835 | 9-Hexadecenoic acid, n | |



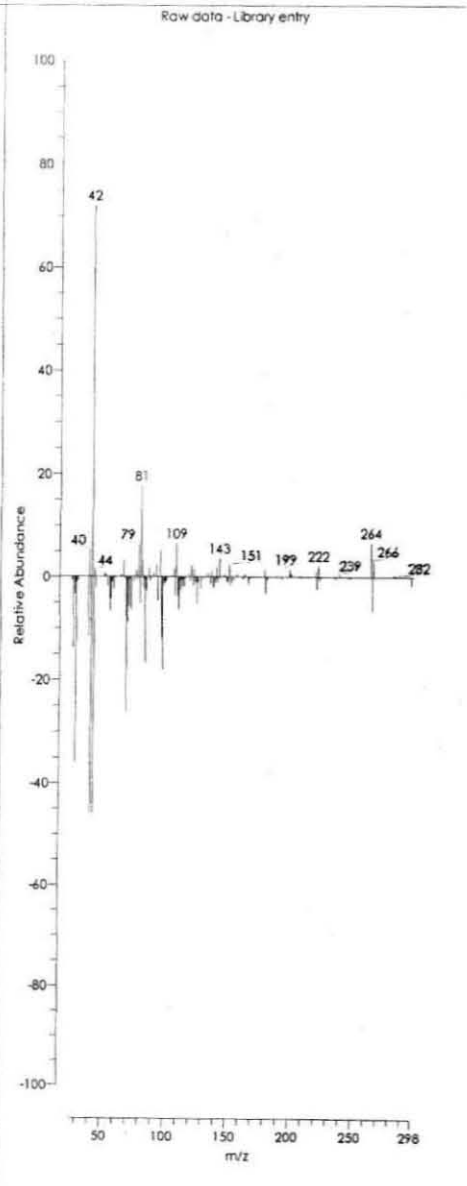




**Fig – 5 Mass spectrum of 3-1-8**

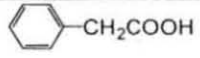
Analysis of fraction **3-2** and **3-3 (Table - 2)**

Compound **3-2-1** (**Fig - 6**) is identified as 2-methylhexadecanol-1 (*m.p.* 60-61 °C). The $M^+$ is absent. The first peak at *m/e* 255 is formed by cleavage of methyl group attached at C-2 atom. Elimination of $[CH_2OH]^{+\cdot}$ unit leads to fragment with *m/e* 225. The cleavage at C-C bonds removed successively from the oxygen atom gives rise to prominent peaks with *m/e* 57, 71, 85 and 97 with $CH_2$ mass unit variance.

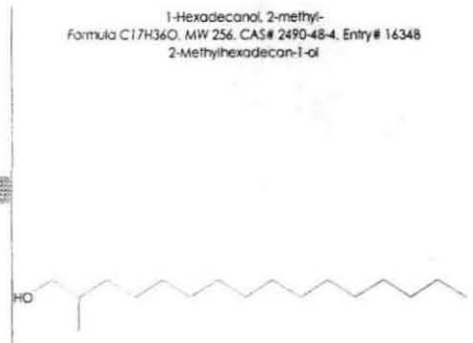
Compound **3-3-1** (**Fig - 7**) is phenylacetic acid. Both M+1 and $M^+$ are present as *m/e* 137 and 136. The BP at 91 shows the formation of tropylium cation from benzyl cation radical. The molecular data are shown in **Table - 2.**

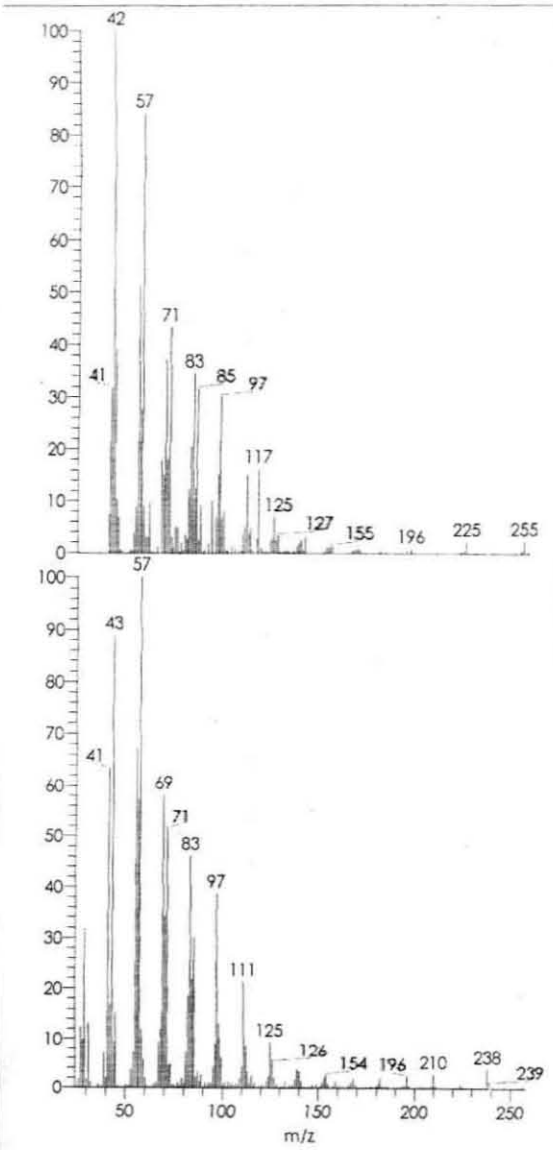
Thus this sponge is housing the array of compounds of methyl esters of long chain fatty acids apart from simple compounds like branched methyl esters, phenylacetic acid and an alcohol in the dichloromethane soluble portion of the alcoholic extract.

**Table - 2**: Analysis of fraction **3-2** and **3-3**

| Compd No. | RT | MF | MW | Structure | 1st MS Peak | Prominent peaks in m/e (1st Peak = Base Peak) |
|---|---|---|---|---|---|---|
| **3-2-1** | 15.31 | $C_{17}H_{36}O$ | 270 |  2-Methylhexadecanol-1 | 255 | 42, 57, 71, 85, 97, 225 |
| **3-3-1** | 4.34 | $C_8H_8O_2$ | 136 |  Phenylacetic aacid | 137 | 91, 42, 65 |

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 42 | 711 | 744 | 1-Docosene | |
| 43 | 711 | 788 | Vinyl lauryl ether | |
| 44 | 709 | 778 | Hexadecanal | |
| 45 | 708 | 734 | Ethanol, 2-(tetradecyloxy)- | |
| 46 | 706 | 750 | 1-Hexadecene | |
| **47** | **706** | **707** | **1-Hexadecanol, 2-methyl-** | |
| 48 | 705 | 727 | 1-Octadecene | |
| 49 | 705 | 722 | 1-Octadecanethiol | |
| 50 | 705 | 745 | Decane, 1,1'-oxybis- | |
| 51 | 704 | 718 | Hexadecane, 1-chloro- | |
| 52 | 704 | 709 | Octadecane, 1-chloro- | |
| 53 | 703 | 760 | Tetradecanal | |
| 54 | 703 | 713 | Ethanol, 2-(hexadecyloxy)- | |
| 55 | 702 | 752 | Silane, trichlorodocosyl- | |
| 56 | 700 | 707 | 1-Hexadecene | |
| 57 | 698 | 708 | Ethanol, 2-(dodecyloxy)- | |
| 58 | 698 | 807 | 2-Hexyl-1-octanol | |
| 59 | 695 | 743 | 2-Trifluoroacetoxytetradecane | |



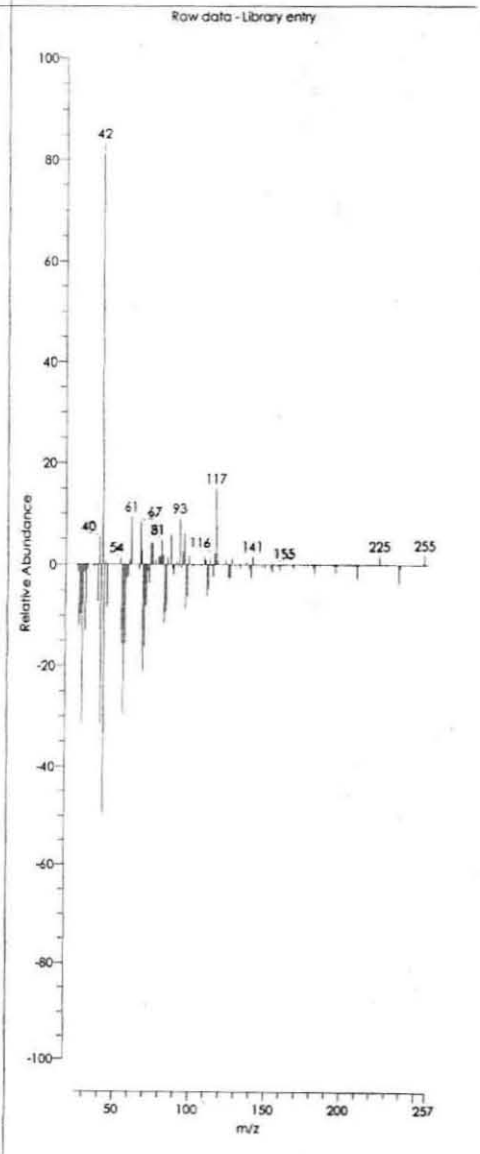
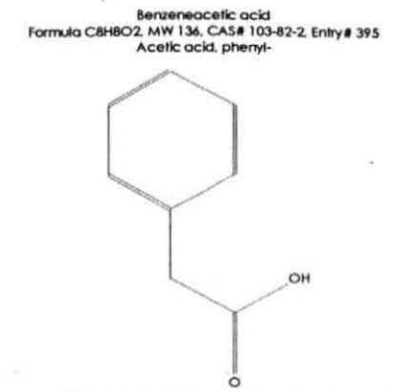
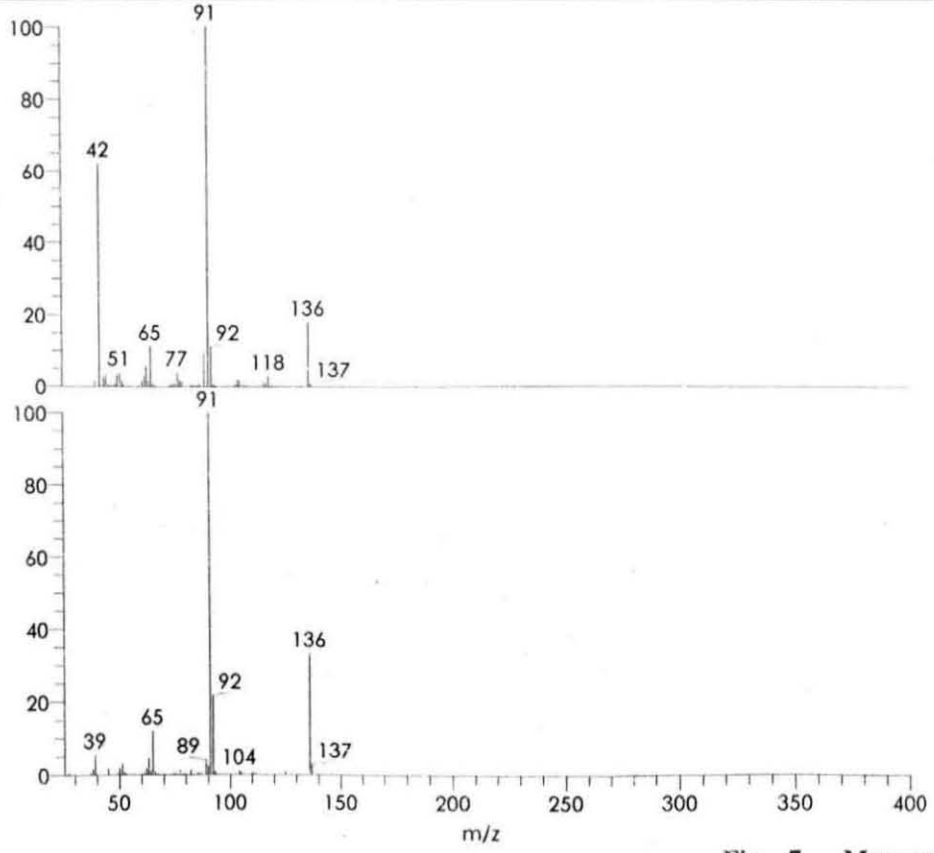




**Fig – 6 Mass spectrum of 3-2-1**

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 783 | 796 | Propane | |
| 2 | 765 | 873 | Benzene | |
| 3 | 750 | 839 | Benzen | |
| 4 | 750 | 839 | Benzene | |
| 5 | 736 | 815 | Benzene | |
| 6 | 716 | 797 | Benzene | |
| 7 | 710 | 752 | 3-Oxo-4 | |
| 8 | 700 | 854 | Thiocya | |
| 9 | 691 | 862 | Benzene | |
| 10 | 668 | 736 | Benzoic | |
| 11 | 667 | 825 | Benzyl c | |
| 12 | 665 | 733 | Benzene | |
| 13 | 665 | 747 | Benzene | |
| 14 | 662 | 736 | Benzene | |



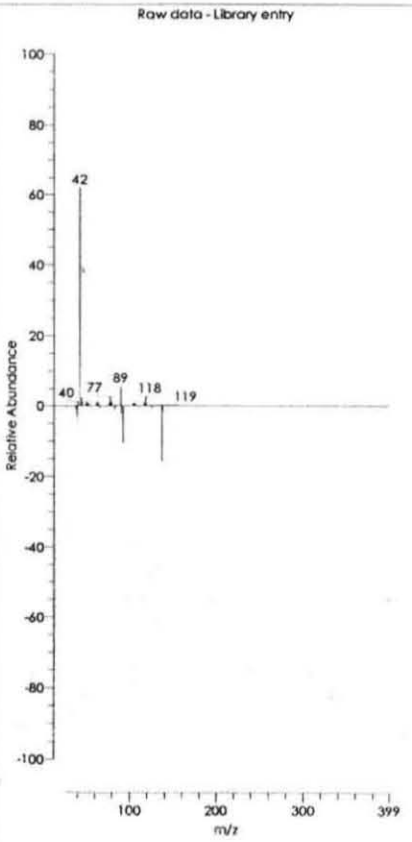




**Fig – 7 Mass spectrum of 3-3-1**

**References**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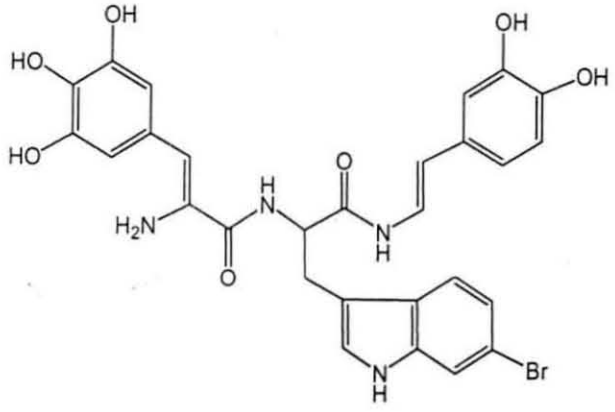
Chapter 4

# Chemical investigation of the sponge *Cervicornia* spp.

**Compounds of the sponge family *Clionidae***

Sponges of the family *Clionidae* are usually burrowing organisms over their substrates such as, corals, rocks and oyster shells. These sponges have been found to contain alkaloids with linear peptide and aromatic substitutions. Anderson *et al.* isolated clionamide[1,2] and celenamide A-D[3,4] from *Cliona celata*. Plumericin was isolated from *C. caribboea.*[5] Recently storniamides A-D, hexacyclic aromatic alkaloids[6] from a Patagonian sponge of the genus *Cliona* sp. and celenamide E (**1**)[7] and a tripeptide alkaloid from *Cliona chilensis*, have been isolated by Palermo *et al.*




1

**Chemical Analysis of Sponge *Cervicornia* sp.**

Classification

| | | |
|---|---|---|
| Phylum | - | Porifera |
| Class | - | Demospongia |
| Sub class | - | Tetractinomorpha |
| Order | - | Hadromerida |
| Family | - | *Clionidae* (D'Orbigny 1852) |
| Genus | - | *Cervicornia* spp. |

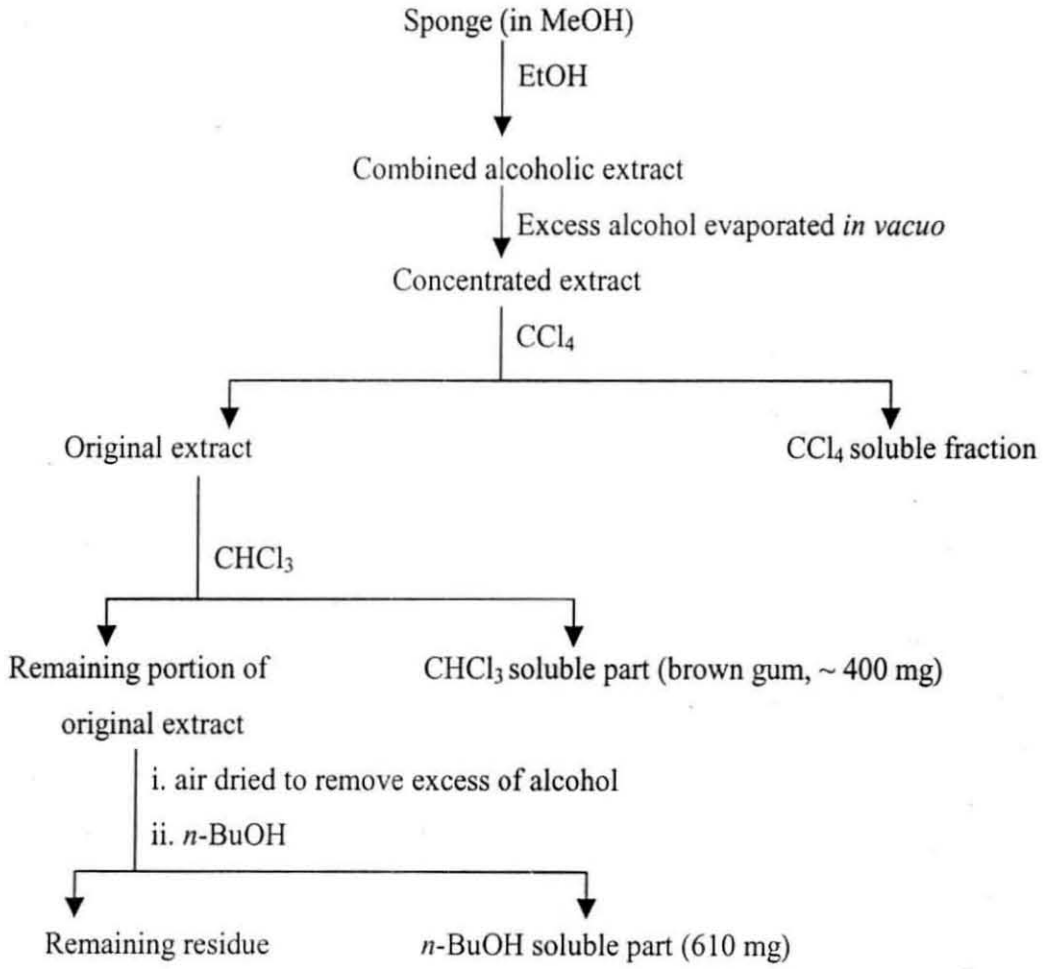
**Fig - 1**

The methanol (~1.5 lit) in which the sponge *Cervicornia* spp. (**Fig - 1**) (~2 kg) was kept immersed, was decanted. The sponge was then further extracted with ethanol and the extract was combined with the methanolic extract. The combined alcoholic extract was fractionated as given in **Scheme - 1**. There has been no report of chemical investigation on this specimen.

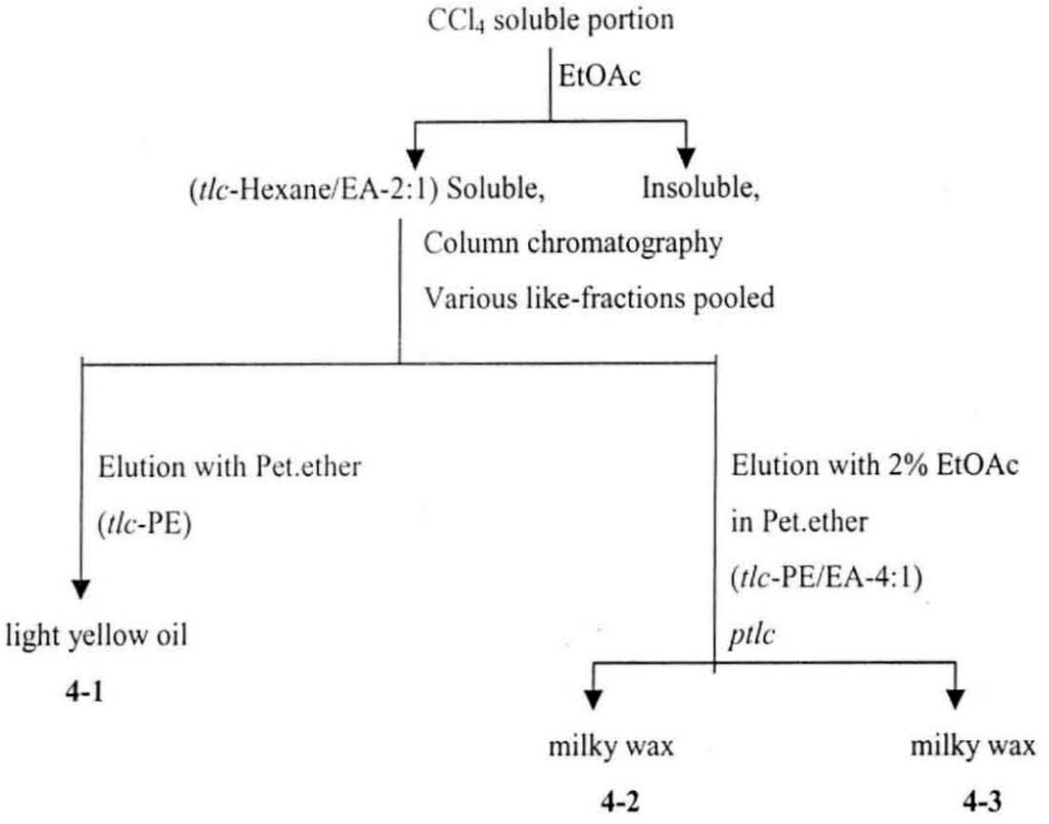
The concentrated alcoholic extract was extracted with carbon tetrachloride, chloroform and *n*-butanol one after another to get the respective extracts. The carbon tetrachloride extract was treated with ethyl acetate to get the ethyl acetate soluble fraction. The remaining waxy mass was discarded. The ethyl acetate soluble portion was then subjected to column chromatography using pet. ether with increasing concentration of ethyl acetate. Three semi pure fractions, **4-1**, **4-2** and **4-3** have been obtained in this

process (**Scheme - 2**). The chloroform soluble fraction and the *n*-butanol soluble fraction do not contain any recognizable organic compounds as revealed by *tlc*.

**Extraction and Fractionation of *Cervicornia* sp.**
(By modified Kupchan method)[8]




**Scheme - 1**




**Scheme - 2**

Analysis of fraction **4-1 (Table - 1)**

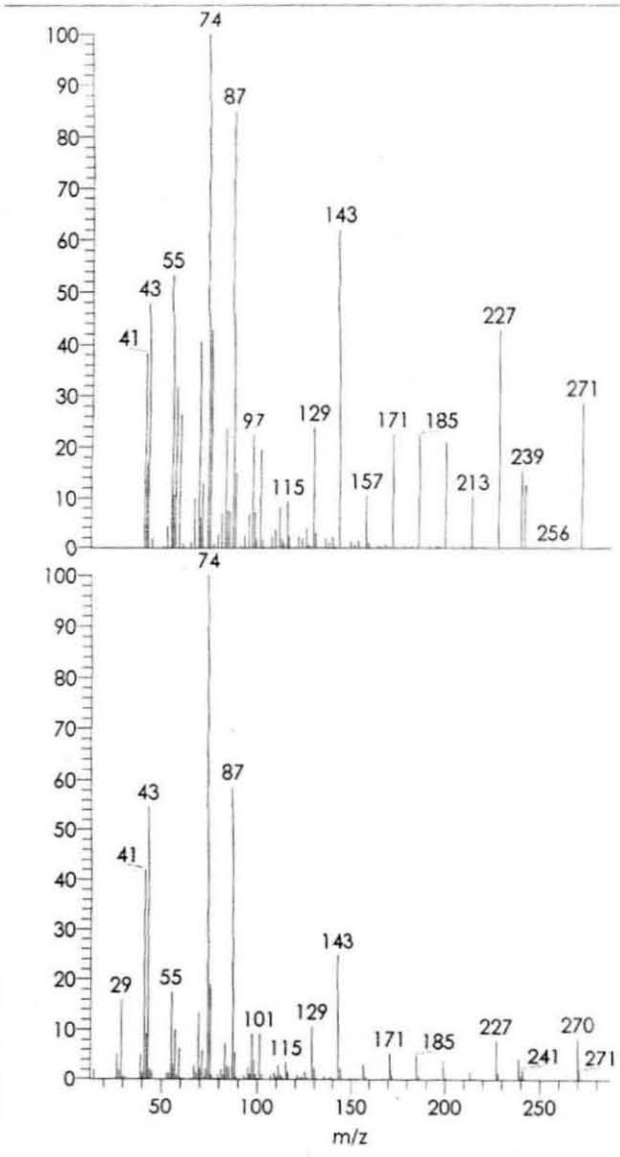
Total of eleven compounds from methyl tetradecanoate to methyl tetracosanoate including a branched $C_{16}$ ester (**4-1-1** to **4-1-11**), were present in this fraction.

Methyl esters of saturated and unsaturated long chain fatty acids have been identified. Both $M^+$ (**4-1-1, 4-1-2, 4-1-4** and **4-1-6**) and M+1 [**4-1-3** (**Fig - 2**), **4-1-5, 4-1-7, 4-1-8** (**Fig - 3**), **4-1-9, 4-1-10** (**Fig - 4**) and **4-1-11**] peaks are present for the compounds. The base peak at *m/e* 74 is present in all the saturated straight chain compounds (**4-1-1, 4-1-3, 4-1-5, 4-1-7, 4-1-9** and **4-1-11**) characteristic of methyl esters arising out of McLafferty rearrangement. Unsaturated esters (**4-1-4, 4-1-8** and **4-1-10**) have base peak at 55 for $[C_4H_7]^{+\cdot}$ unit. The mass spectrum has the clustered peaks with successive mass difference of 14 accountable for $CH_2$ are seen for unsaturated fatty esters. These clustered peaks are prevalent in saturated fatty acid esters after *m/e* 129.

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 798 | 800 | Hexadecanc | |
| 2 | 798 | 801 | Hexadecanc | |
| 3 | 775 | 788 | Hexadecanc | |
| 4 | 773 | 781 | Hexadecanc | |
| 5 | 728 | 728 | Hexadecanc | |
| 6 | 718 | 769 | 9-Octadecer | |
| 7 | 695 | 747 | Heptadecan | |
| 8 | 695 | 763 | Pentadecan | |
| 9 | 694 | 710 | Pentadecan | |
| 10 | 685 | 733 | Eicosanoic a | |
| 11 | 684 | 687 | Pentadecan | |
| 12 | 680 | 729 | Octadecanc | |
| 13 | 665 | 672 | Hexadecanc | |
| 14 | 661 | 713 | Heneicosanc | |
| 15 | 657 | 705 | Heptadecan | |
| 16 | 654 | 781 | Tridecanoic c | |
| 17 | 648 | 648 | Pentadecan | |
| 18 | 644 | 735 | Methyl tetrac | |

Hexadecanoic acid, methyl ester

Palmitic acid, methyl ester



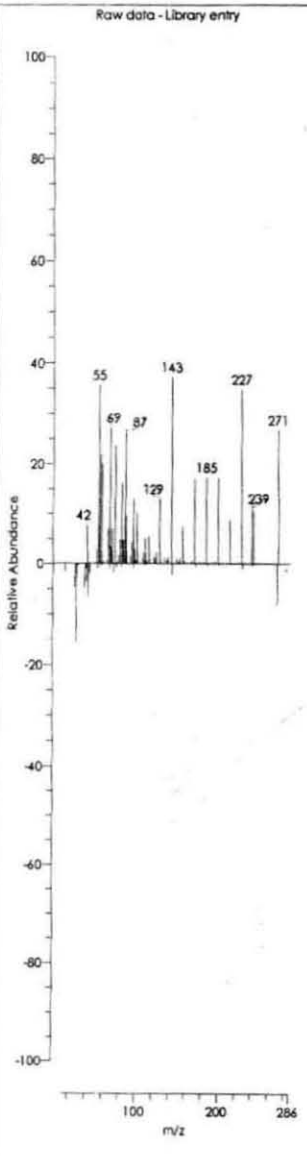




**Fig – 2 Mass spectrum of 4-1-3**

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 751 | 756 | 13-Docosenc | |
| 2 | 703 | 808 | 9-Octadecer | |
| 3 | 703 | 808 | 9-Octadecer | |
| 4 | 697 | 855 | 9-Octadecer | |
| 5 | 686 | 817 | Triolein | |
| 6 | 680 | 684 | Erucic acid | |
| 7 | 673 | 683 | Erucic acid | |
| 8 | 670 | 795 | 9-Hexadecer | |
| 9 | 668 | 821 | 9-Octadecer | |
| 10 | 667 | 780 | 13-Docosenc | |
| 11 | 665 | 816 | 9-Octadecer | |
| 12 | 659 | 662 | 13-Docosenc | |
| 13 | 659 | 746 | 9-Octadecer | |
| 14 | 656 | 737 | Cyclopropar | |
| 15 | 653 | 669 | Erucic acid | |
| 16 | 651 | 748 | 9-Octadecer | |
| 17 | 650 | 734 | 11-Octadece | |
| 18 | 649 | 769 | 9-Octadecer | |

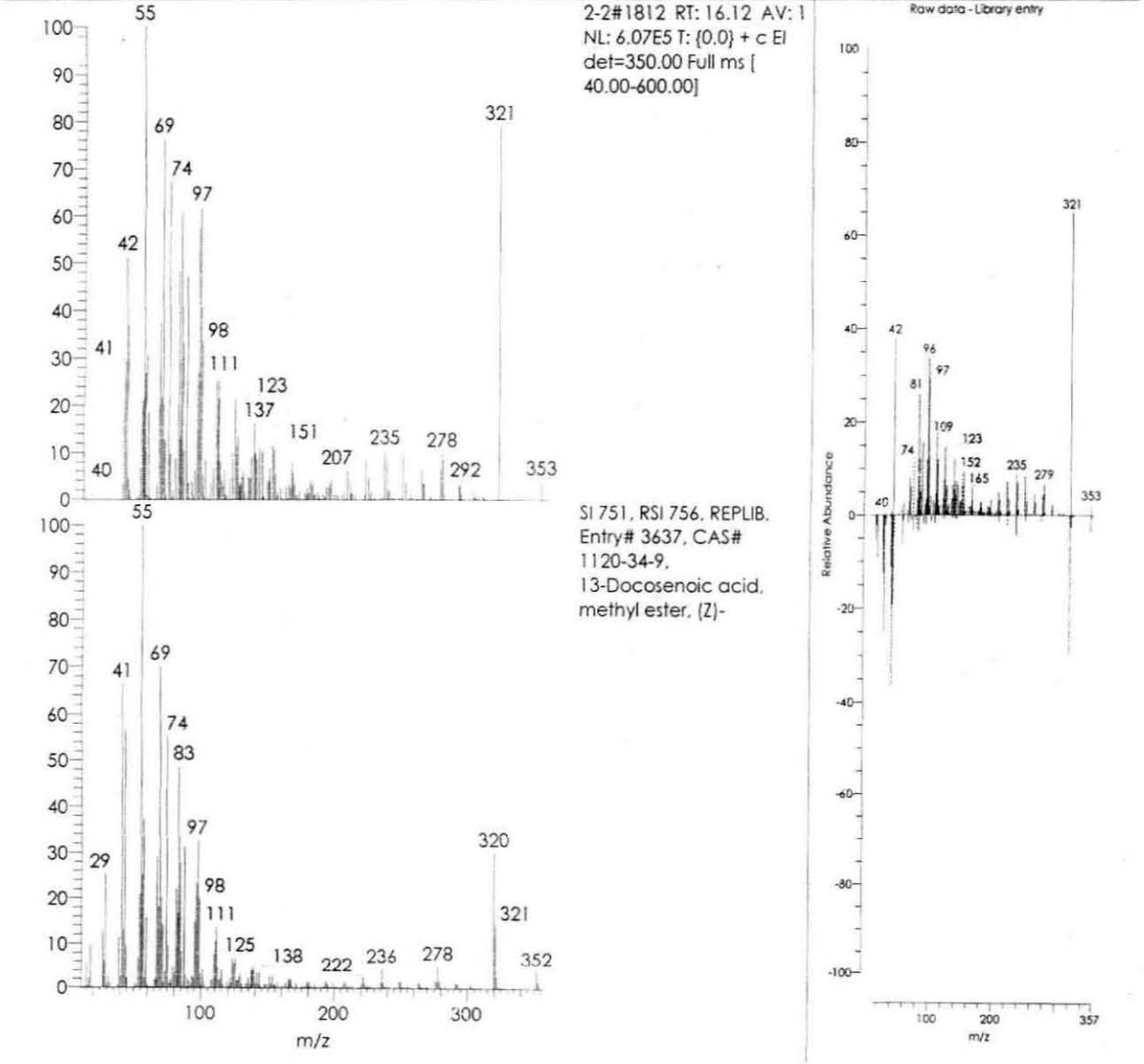
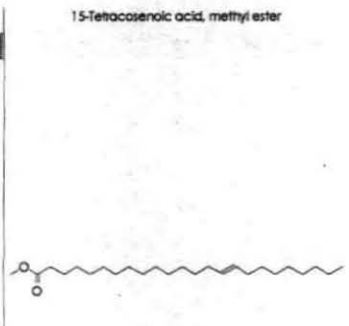
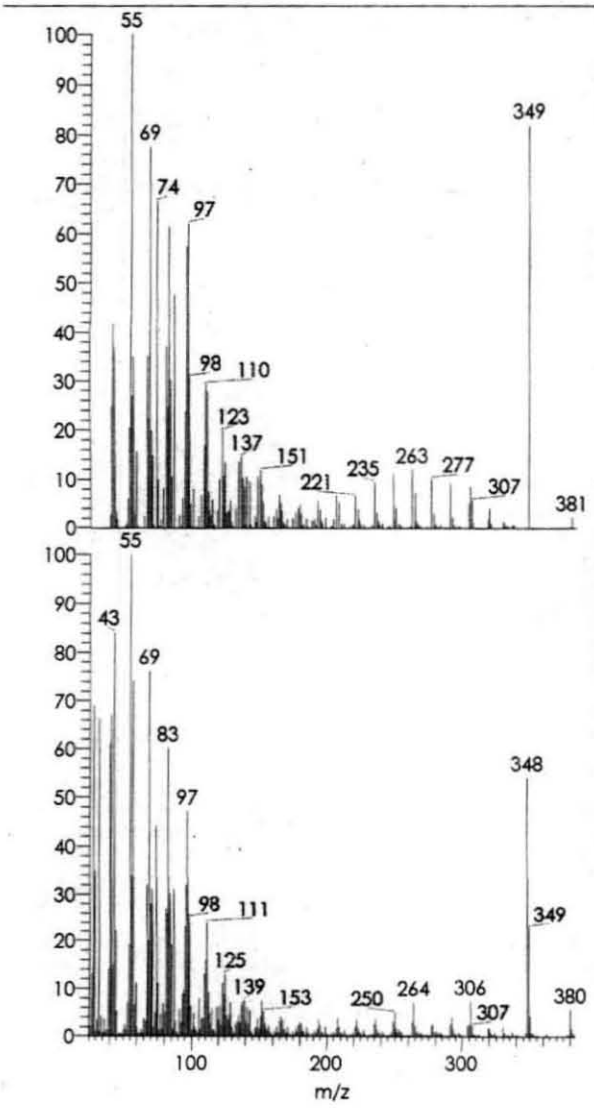




**Fig – 3 Mass spectrum of 4-1-8**

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 656 | 656 | 15-Tetracose | |
| 2 | 654 | 657 | 15-Tetracose | |
| 3 | 652 | 730 | 13-Docosenc | |
| 4 | 652 | 831 | 9-Octadecer | |
| 5 | 645 | 678 | 15-Tetracose | |
| 6 | 637 | 775 | 9-Octadecer | |
| 7 | 637 | 775 | 9-Octadecer | |
| 8 | 630 | 791 | Triolein | |
| 9 | 626 | 795 | 9-Octadecer | |
| 10 | 625 | 720 | Erucic acid | |
| 11 | 624 | 696 | Erucic acid | |
| 12 | 623 | 792 | 9-Octadecer | |
| 13 | 614 | 729 | 9-Octadecer | |
| 14 | 613 | 828 | 13-Docosenc | |
| 15 | 608 | 719 | 11-Octadece | |
| 16 | 607 | 697 | Oleic Acid | |
| 17 | 602 | 680 | Erucic acid | |
| 18 | 602 | 708 | 9-Octadecer | |




2-2#2009 RT: 17.81 AV: 1 NL: 7.07E5 T: {0,0} + c EI det=350.00 Full ms [ 40.00-600.00]




SI 656, RSI 656, REPLIB, Entry# 3555, CAS# 56554-33-7, 15-Tetracosenoic acid, methyl ester

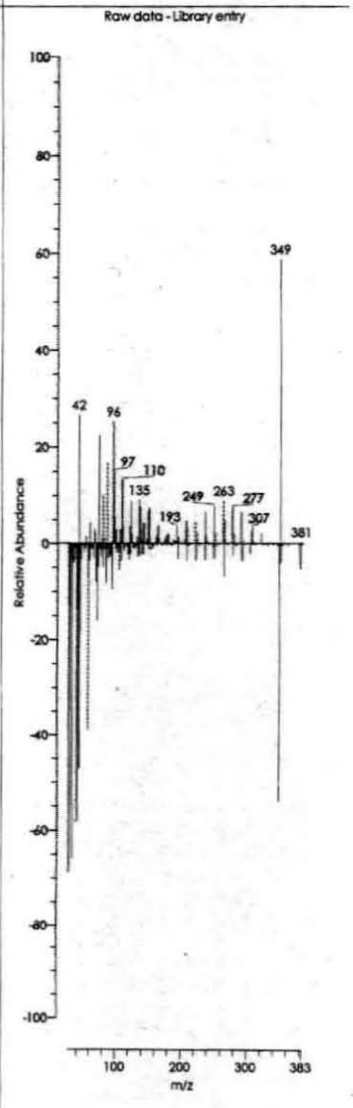



**Fig – 4 Mass spectrum of 4-1-10**

The composition of methyl hexadecanoate, **4-1-3** is the highest followed by that of methyl oleate, **4-1-4**, while that of other compounds are below 5%. The branched methyl ester **4-1-2** is only 1.52% in contrast to the fraction **3-3** of *Siphonochalina* spp. Methyl esters of higher homologues from $C_{19}$ to $C_{24}$ fatty acids are present. Other data depicting RT, percentage composition, molecular formula, molecular weight, structure, prominent mass spectral peaks with a base peak of all the compounds of this fraction are given in **Table - 1**. Compounds (**4-1-6, 4-1-9** and **4-1-11**) are present in traces. From the table, the comparative picture of all eleven compounds are apparent.

Analysis of fraction **4-2 (Table - 2)**

Eight compounds *viz.* **4-2-1** to **4-2-8** were present in this fraction **(Table - 2)**. Five compounds [**4-2-1, 4-2-2** (**Fig - 5**), **4-2-3, 4-2-4** and **4-2-5**] are saturated free long chain fatty acids with chain length from $C_{14}$ to $C_{20}$. The $C_{16}$ acid contributes the maximum composition of 63.2%. Compound **4-2-6** (**Fig - 6**) is a phthalate derivative and the rest **4-2-7** and **4-2-8** (**Fig - 7**) are steroids. Being the higher polar fraction when compared with **4-1**, it has no methyl ester. Less than $1/4^{th}$ of fraction is contributed by the steroids *viz.* cholesterol (10.63%) and $\tau$-sitosterol (10.63%).

The peak intensity of fragments at *m/e* 60 and 73 are nearly equal for **4-2-1, 4-2-2, 4-2-3** and **4-2-4** with base peak at 60. The clustered fragments with mass unit difference are characteristic of unbranched straight chain type. Elimination of acetic acid with *m/e* 60 is evident in all the acids. The cleavage at $\beta$-$\gamma$ bond resulting in the fragment ion $[CH_2CH_2COOH]^{+\cdot}$ is also dominant. The cleavage at $C_7$-$C_8$ bond leading to carboxyl containing unit having *m/e* 129 is prevalent in all the acids. Compound **4-2-6** is diisooctyl phthalate. Elimination of one $C_8H_{17}$ alkyl unit gives *m/e* 280 as the first mass spectral peak and cleavage of $2^{nd}$ alkyl unit gives the peak at 167. Formation of phthalic anhydride giving *m/e* 169 is typical of phthalate derivatives. The presence of isooctyl group is evident by the ready formation of the fragment *m/e* 57 resulted by the cleavage at $C_3$-$C_4$ bond leading to isobutyl and *n*-butyl units.

Table - 1: Analysis of fraction **4-1**

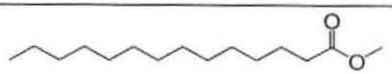
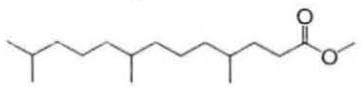
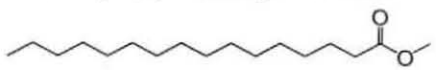
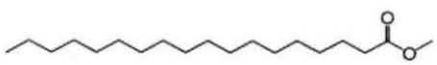
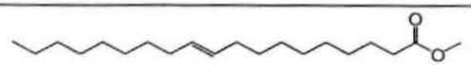
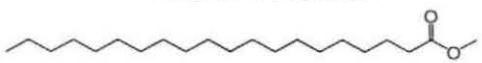
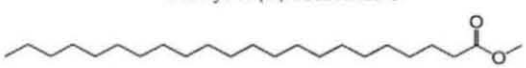
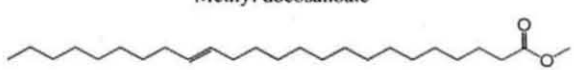
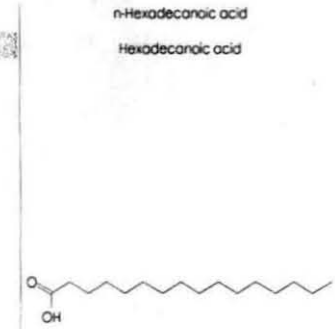
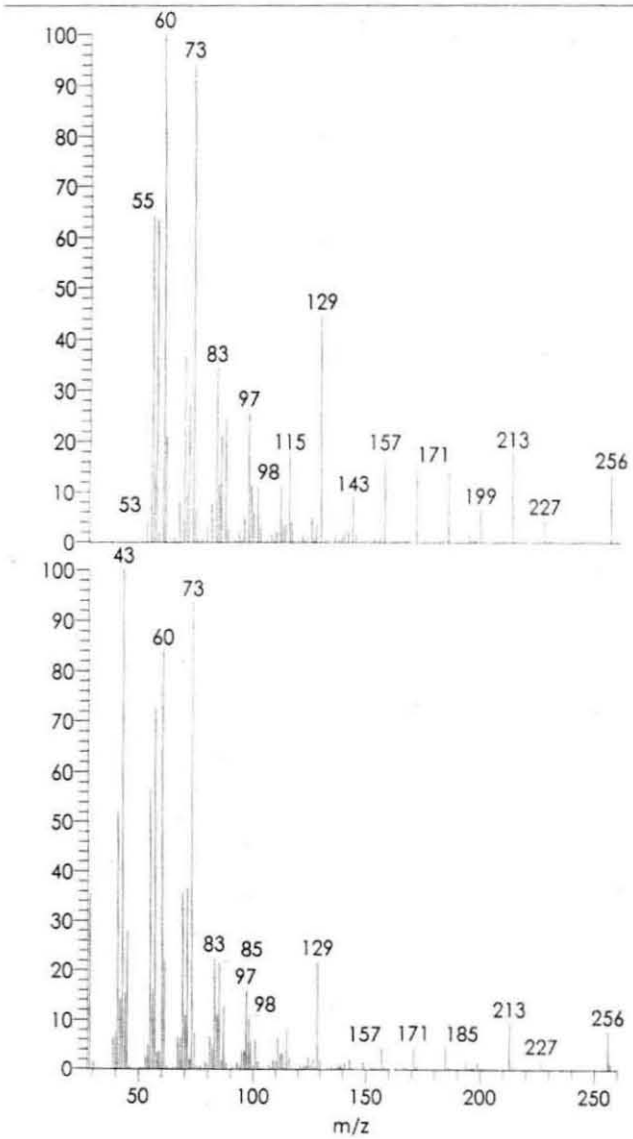
| Compd No. | RT | Peak area %age | MF | MW | Structure | 1st ms peak | Prominent peaks in *m/e* (1st Peak = Base Peak) |
|---|---|---|---|---|---|---|---|
| **4-1-1** | 8.76 | 4.68 | $C_{15}H_{30}O_2$ | 242 |  Methyl tetradecanoate | 242 | 74, 87, 43, 57 |
| **4-1-2** | 9.25 | 1.52 | $C_{17}H_{34}O_2$ | 270 |  Methyl 4,8, 12-trimethyl tridecanoate | 270 | 87, 74, 57, 113, 157, 213 |
| **4-1-3** | 11.09 | 42.87 | $C_{17}H_{34}O_2$ | 270 |  Methyl hexadecanoate | 271 | 74, 87, 42, 55, 43, 143, 227 |
| **4-1-4** | 12.75 | 35.08 | $C_{19}H_{36}O_2$ | 296 |  Methyl oleate | 296 | 55, 42, 74, 83, 96, 110, 222, 264 |
| **4-1-5** | 12.88 | 2.97 | $C_{19}H_{38}O_2$ | 298 |  Methyl octadecanoate | 299 | 74, 87, 42, 55, 143, 97, 255 |

**Table – 1 Contd...**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **4-1-6** | 12.93 | 0.73 | $C_{20}H_{38}O_2$ | 310 |  Methyl 10-nonadecenoate | 310 | 69, 55, 83, 139, 97, 125, 171, 194, 280 |
| **4-1-7** | 14.62 | 2.77 | $C_{21}H_{42}O_2$ | 326 |  Methyl eicosanoate | 327 | 74, 87, 143, 55, 283 |
| **4-1-8** | 16.12 | ~2.36 | $C_{23}H_{44}O_2$ | 352 |  Methyl 13(Z)-docosenoate | 353 | 55, 69, 74, 83, 97, 321, 42,111, 123, 137 |
| **4-1-9** | 16.24 | ~0.20 | $C_{23}H_{46}O_2$ | 354 |  Methyl docosanoate | 355 | 74, 42, 87, 143, 55, 312 |
| **4-1-10** | 17.81 | 2.99 | $C_{25}H_{48}O_2$ | 380 |  Methyl 15-tetracosenoate | 381 | 55, 349, 69, 74, 97, 110 |
| **4-1-11** | 17.93 | ~0.20 | $C_{25}H_{50}O_2$ | 382 |  Methyl tetracosanoate | 384 | 123, 74, 87, 42, 55, 143 |

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 834 | 835 | n-He | |
| 2 | 816 | 817 | n-He | |
| 3 | 816 | 817 | n-He | |
| 4 | 815 | 815 | n-He | |
| 5 | 813 | 814 | n-He | |
| 6 | 774 | 851 | Tetrc | |
| 7 | 774 | 851 | Tetrc | |
| 8 | 773 | 825 | Hept | |
| 9 | 763 | 801 | Tetrc | |
| 10 | 752 | 785 | Pent | |
| 11 | 746 | 773 | Tetrc | |
| 12 | 739 | 740 | Octc | |
| 13 | 723 | 752 | Pent | |
| 14 | 718 | 807 | Tride | |
| 15 | 710 | 784 | Tride | |
| 16 | 710 | 784 | Tride | |
| 17 | 710 | 871 | Unde | |
| 18 | 708 | 708 | Octc | |



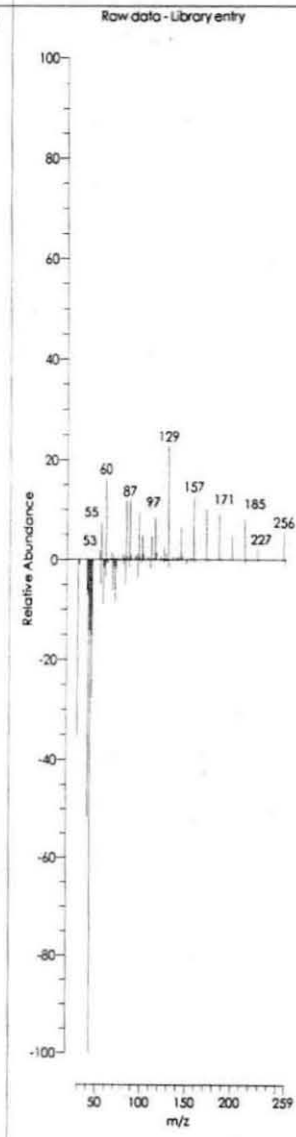
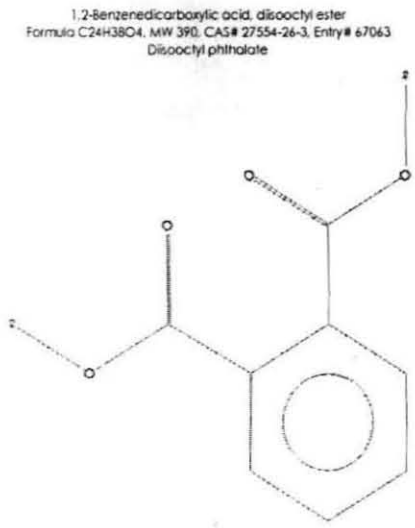
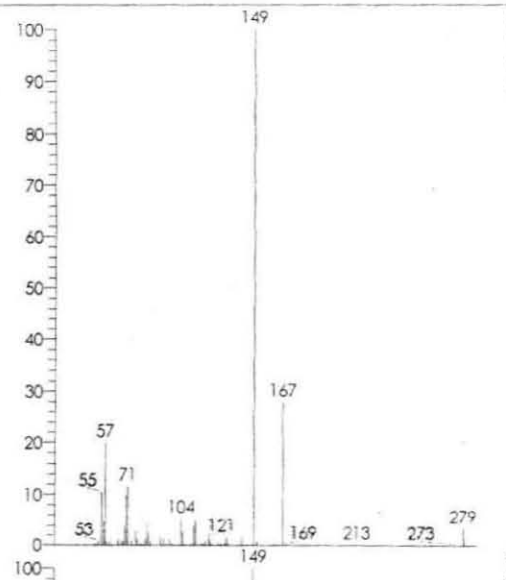



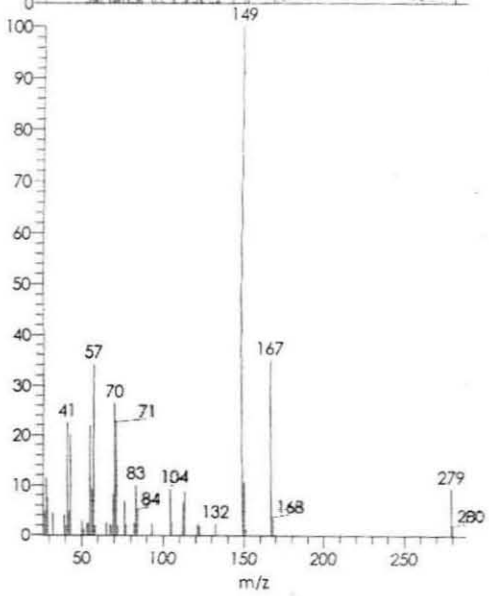

**Fig – 5 Mass spectrum of 4-2-2**

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 854 | 885 | 1,2-Benzenedicarboxylic a | |
| 2 | 837 | 867 | Bis(2-ethylhexyl) phthalate | |
| 3 | 837 | 867 | Bis(2-ethylhexyl) phthalate | |
| 4 | 823 | 859 | Bis(2-ethylhexyl) phthalate | |
| 5 | 820 | 831 | 1,2-Benzenedicarboxylic ac | |
| 6 | 817 | 826 | Di-n-octyl phthalate | |
| 7 | 802 | 862 | Bis(2-ethylhexyl) phthalate | |
| 8 | 792 | 803 | Bis(2-ethylhexyl) phthalate | |
| 9 | 784 | 790 | Di-n-octyl phthalate | |
| 10 | 781 | 832 | Phthalic acid, diisooctyl est | |
| 11 | 771 | 793 | Di-n-octyl phthalate | |
| 12 | 771 | 793 | Di-n-octyl phthalate | |
| 13 | 749 | 761 | 1,2-Benzenedicarboxylic ac | |
| 14 | 743 | 758 | 1,2-Benzenedicarboxylic ac | |
| 15 | 726 | 734 | 1,2-Benzenedicarboxylic ac | |
| 16 | 721 | 736 | 1,2-Benzenedicarboxylic ac | |
| 17 | 715 | 725 | Di-n-octyl phthalate | |
| 18 | 702 | 775 | Didodecyl phthalate | |




2-3#2048 RT: 16.44 AV: 1 NL: 9.25E5 T: {0,0} + c EI det=350.00 Full ms [ 50.00-550.00]




SI 854, RSI 885, MAINLIB, Entry# 67063, CAS# 27554-26-3, 1,2-Benzenedicarboxylic acid, diisooctyl ester

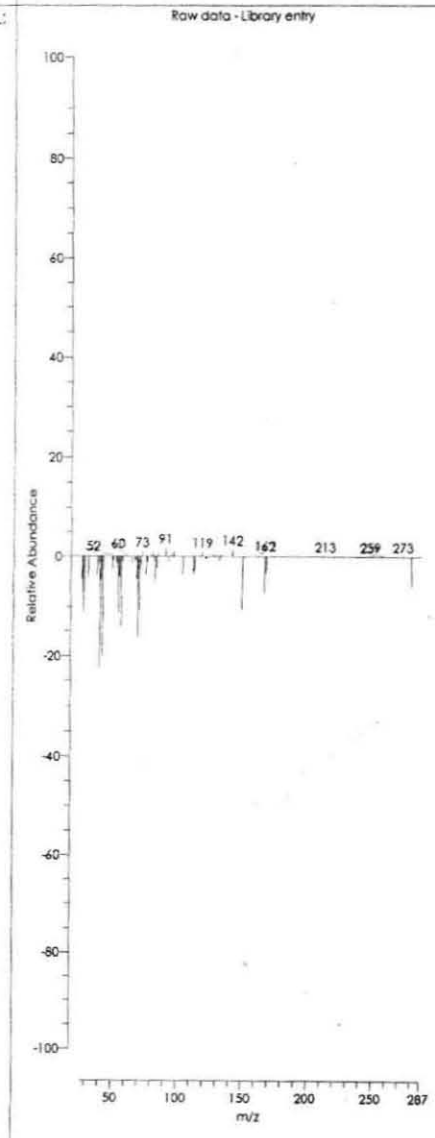
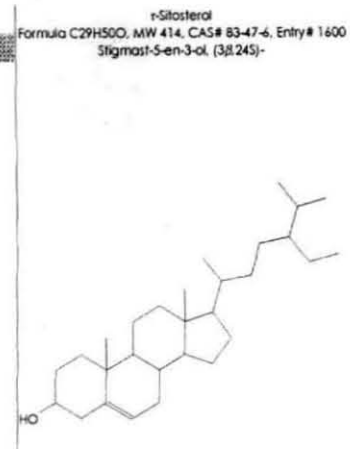
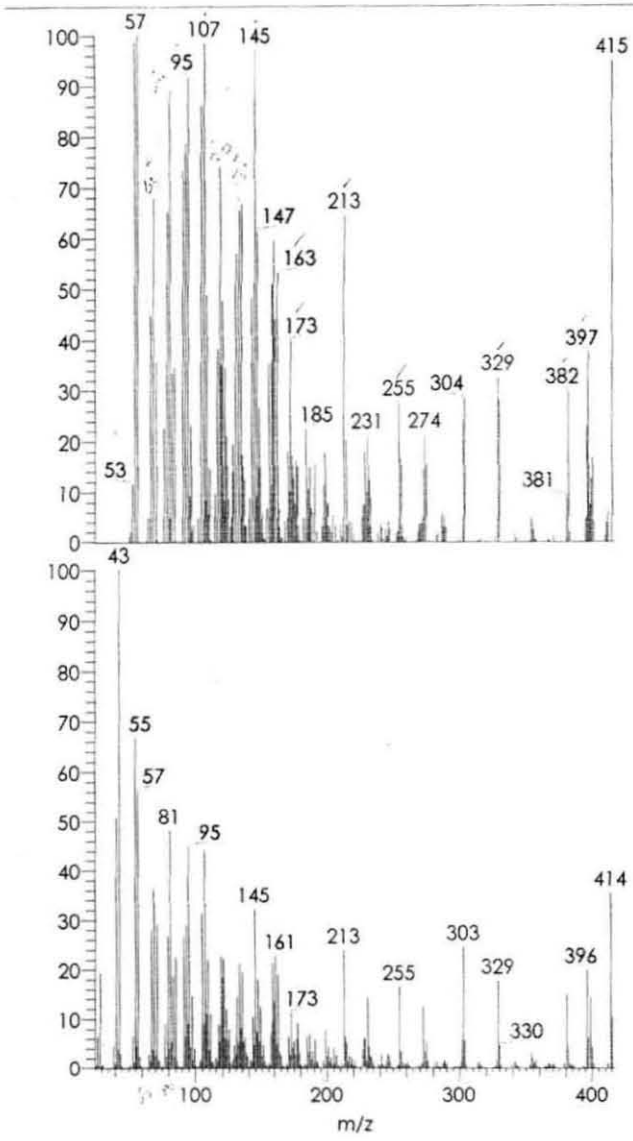



**Fig – 6 Mass spectrum of 4-2-6**

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 714 | 720 | τ-Sitosterol | |
| 2 | 678 | 773 | τ-Sitosterol | |
| 3 | 659 | 665 | β-Sitosterol | |
| 4 | 648 | 652 | Stigmasterol, | |
| 5 | 645 | 645 | β-Sitosterol | |
| 6 | 644 | 649 | β-Sitosterol | |
| 7 | 639 | 676 | β-Sitosterol ac | |
| 8 | 631 | 779 | Campesterol | |
| 9 | 620 | 698 | Campesterol | |
| 10 | 618 | 644 | β-Sitosterol | |
| 11 | 608 | 666 | β-Sitosterol ac | |
| 12 | 597 | 643 | 5-Cholestene | |
| 13 | 591 | 653 | Cholesterol | |
| 14 | 585 | 616 | Cholest-5-en- | |
| 15 | 581 | 641 | Stigmast-5-er | |
| 16 | 573 | 691 | Ergost-5-en-3- | |
| 17 | 572 | 694 | Cholesterol | |
| 18 | 572 | 694 | Cholesterol | |



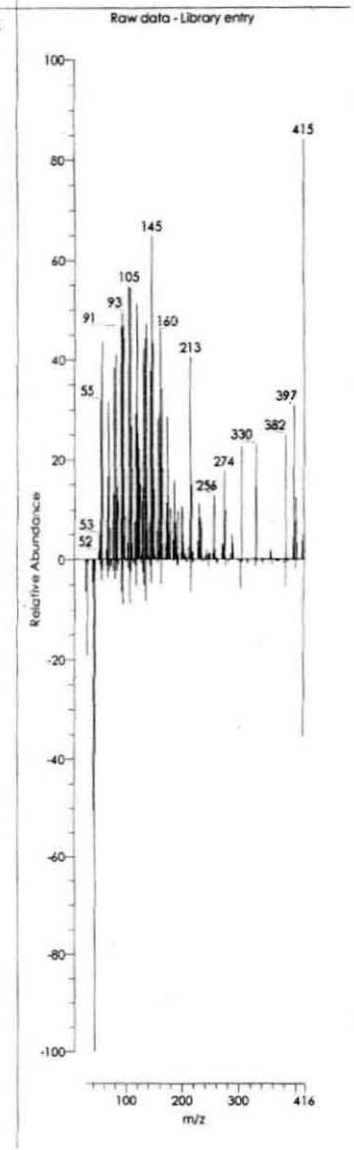




**Fig – 7 Mass spectrum of 4-2-8**

Compound **4-2-7** is cholesterol with the base peak at *m/e* 95. The mass spectrum is well matching with that of standard. So is the case with τ-sitosterol (**4-2-8**) also.

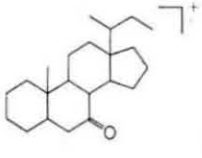
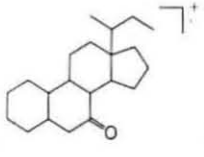
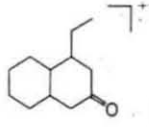
The details of RT, percentage, molecular structure and prominent mass spectral data of the compounds of this fraction are given in **Table - 2**.

Analysis of fraction **4-3 (Table - 3)**

This fraction also contains both free fatty acids of chain length $C_{14}$ and $C_{16}$ **4-3-1** and **4-3-2**, phthalate derivative **4-3-3** and steroidal derivatives **4-3-4** to **4-3-10**. The steroidal derivatives form major composition (~ 92%) leaving fatty acids and phthalate derivative of only ~ 8%. Stigmasta-3,5-dien-7-one, **4-3-10** (**Fig - 9**) contributes highest composition of 24% followed by derivative **4-3-8** (**Fig - 8**) with 22%. Steroidal derivatives **4-3-4**, **4-3-5, 4-3-6, 4-3-7, 4-3-8** and **4-3-9** have the composition range from 5 to 17 %.

Compounds **4-3-10** has base peak at 174. Compounds **4-3-4** and **4-3-5** are cholesta-4,6-dien-3-ol and stigmasta-4,6-dien-3-ol. The alcohols **4-3-4, 4-3-5** and **4-3-6** lose water molecule to give first peak at (M-18).

Fragments of **4-3-9**, stigmastan-7-one, are shown here:

$M-CH_3$ (396),  (329),  (301) and  (179).

The GC-MS data of all the compounds are listed in **Table - 3**.

Thus the sponge, *Cervicornia* spp. has methyl esters of long chain fatty acids, free fatty acids and steroidal derivatives as the components and no hydrocarbons as seen in *Siphonochalina* spp.

**Table - 2:** Analysis of fraction **4-2**

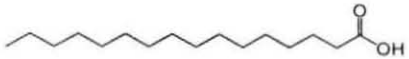
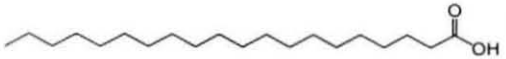
| Comd No. | RT | Peak area %age | MF | MW | Structure | 1st MS Peak | Prominent Peak (1st value BP) |
|---|---|---|---|---|---|---|---|
| **4-2-1** | 9.48 | 1.05 | $C_{14}H_{28}O_2$ | 228 |  Tetradecanoic acid | 229 | 73, 60, 55, 129, 83, 97, 115, 185 |
| **4-2-2** | 11.85 | 63.20 | $C_{16}H_{32}O_2$ | 256 |  Hexadecanoic acid | 256 | 60, 73, 83, 97, 115, 129, 171, 213, 227 |
| **4-2-3** | 12.46 | 1.97 | $C_{17}H_{34}O_2$ | 270 |  Heptadecanoic acid | 271 | 60, 73, 124, 83, 97, 171, 185, 227 |
| **4-2-4** | 13.38 | 5.36 | $C_{18}H_{36}O_2$ | 284 |  Octadecanoic acid | 285 | 60, 73, 55, 83, 129, 97, 185, 171 |
| **4-2-5** | 15.08 | 3.76 | $C_{20}H_{40}O_2$ | 312 |  Eicosanoic acid | 313 | 55, 73, 97, 115, 124, 185 |

**Table – 2 Contd...**

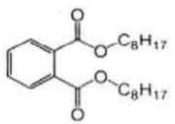
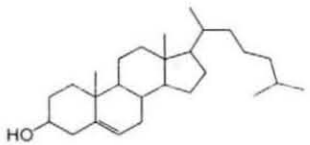
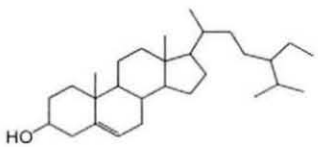
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **4-2-6** | 16.44 | 1.89 | $C_{24}H_{38}O_4$ | 390 |  1,2-Dioctyl benzenedicarboxylate | 280 | 149, 167, 57, 71 |
| **4-2-7** | 23.98 | 11.43 | $C_{27}H_{46}O$ | 386 |  Cholesterol | 388 | 95, 147, 81, 106, 55, 133, 163, 213, 301, 369, 227, 269 |
| **4-2-8** | 29.71 | 10.63 | $C_{29}H_{50}O$ | 414 |  T-Sitosterol | 415 | 107, 145, 95, 120, 81, 65, 135, 163, 173 |

**Table 3:** Analysis of fraction **4-3**

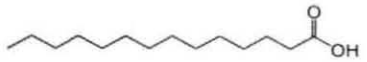
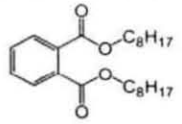
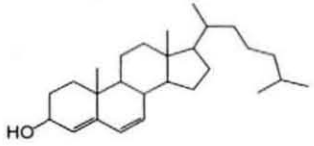
| Comd No. | RT | Peak area %age | MF | MW | Structure | 1st MS Peak | Prominent Peak (1st value BP) |
|---|---|---|---|---|---|---|---|
| **4-3-1** | 9.40 | 0.251 | $C_{14}H_{28}O_2$ | 228 |  Tetradecanoic acid | 229 | 73, 60, 55, 129, 83, 97, 115, 185 |
| **4-3-2** | 11.60 | 7.37 | $C_{16}H_{32}O_2$ | 256 |  Hexadecanoic acid | 256 | 42, 60, 70, 129 |
| **4-3-3** | 16.43 | 0.273 | $C_{24}H_{38}O_4$ | 390 |  1,2-Dioctyl benzenedicarboxylate | 280 | 149, 167, 57, 71 |
| **4-3-4** | 19.59 | 7.42 | $C_{27}H_{44}O$ | 384 |  Cholesta-4,6-dien-3-ol(3B) | 367 | 143, 135, 81, 95, 157, 247, 57, 158 |

**Table – 3 Contd...**

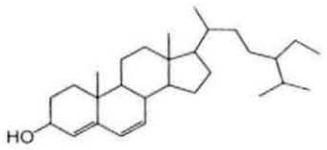
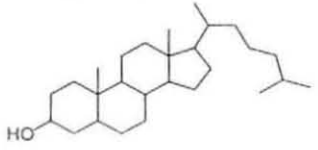
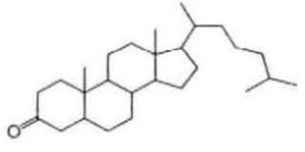
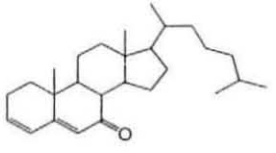
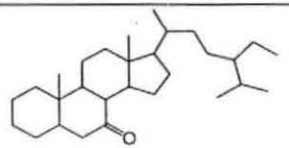
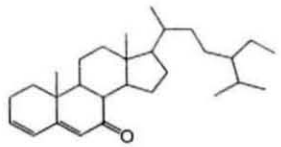
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **4-3-5** | 22.81 | 4.58 | $C_{29}H_{48}O$ | 412 |  Stigmasta-4,6-dien-3-ol | 395 | 143, 135, 81, 95, 157, 247, 57, 158 |
| **4-3-6** | 24.31 | 16.47 | $C_{27}H_{48}O$ | 388 |  Cholestan-3-ol | 389 | 215, 233, 107, 95, 81, 121, 55,165, 374 |
| **4-3-7** | 25.12 | 7.46 | $C_{27}H_{46}O$ | 386 |  Cholestan-3-one | 387 | 231, 55, 81, 95, 217, 107, 163 |
| **4-3-8** | 26.62 | 21.48 | $C_{27}H_{42}O$ | 382 |  Cholesta-3,5-dien-7-one | 383 | 174, 161, 187, 134, 91, 368, 269 |

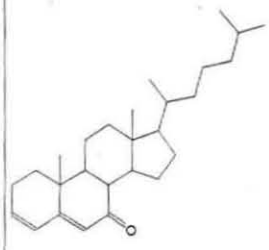
**Table – 3 Contd...**

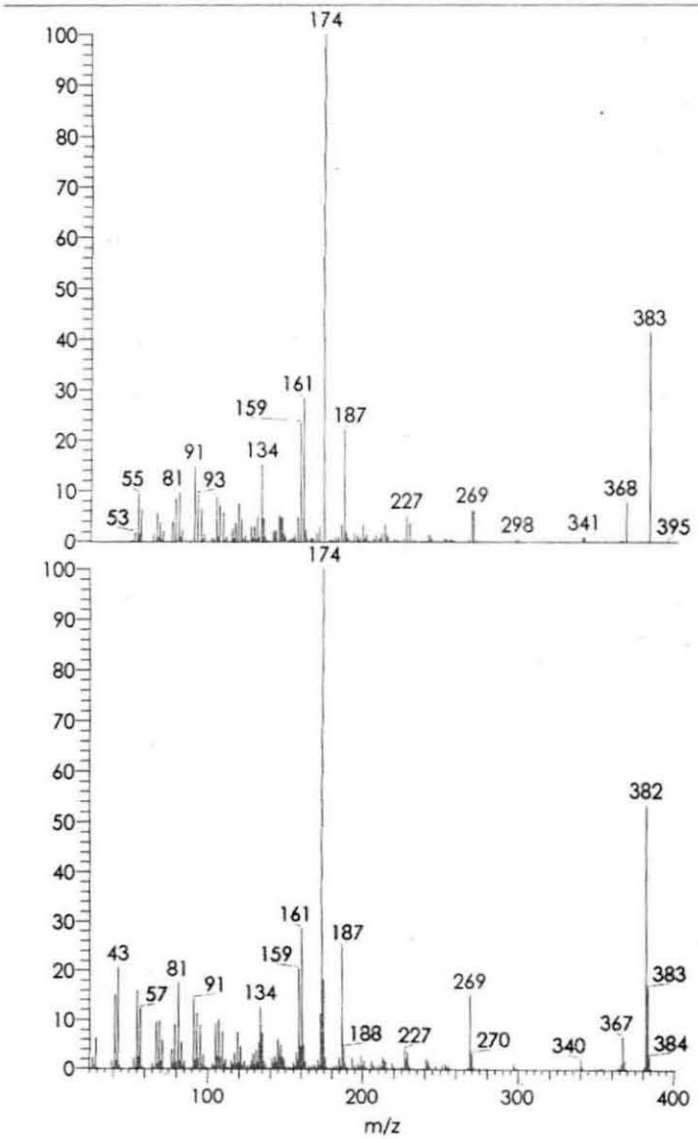
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **4-3-9** | 29.93 | 10.45 | $C_{29}H_{50}O$ | 414 |  Stigmastan-7-one | 415 | 396, 329, 301, 179 |
| **4-3-10** | 34.03 | 24.24 | $C_{29}H_{46}O$ | 410 |  Stigmasta-3,5-dien-7-one | 411 | 174, 161, 187, 269, 91, 134 |

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 666 | 666 | Cholesta-3,5-d | |
| 2 | 647 | 657 | Cholesta-3,5-d | |
| 3 | 629 | 638 | Cholesta-3,5-d | |
| 4 | 556 | 558 | Cholesta-4,6-d | |
| 5 | 512 | 584 | Stigmasta-3,5-d | |
| 6 | 494 | 599 | Androst-5-en-7 | |
| 7 | 492 | 599 | Androsta-3,5-d | |
| 8 | 489 | 552 | Retinol | |
| 9 | 483 | 553 | Retinal, 9-cis- | |
| 10 | 469 | 526 | Corticosterone | |
| 11 | 467 | 477 | 17-(1,5-Dimeth | |
| 12 | 462 | 479 | 9,10-Secochol | |
| 13 | 461 | 463 | b(9a)-Homo-1 | |
| 14 | 455 | 544 | Oxymetholone | |
| 15 | 441 | 490 | Corticosterone | |
| 16 | 436 | 492 | Pregn-5-en-20- | |
| 17 | 432 | 497 | 17α-Methyltest | |
| 18 | 432 | 487 | Androst-5-en-3 | |

Cholesta-3,5-dien-7-one

δ3,5-Cholestadien-7-one






2-4#3355 RT: 26.62 AV: 1 NL: 1.63E6 T: {0,0} + c EI det=350.00 Full ms [ 50.00-550.00]

SI 666, RSI 666, REPLIB, Entry# 17184, CAS# 567-72-6, Cholesta-3,5-dien-7-one

Raw data - Library entry

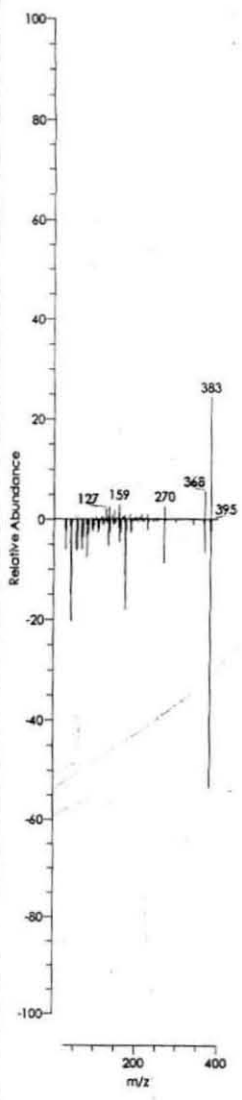
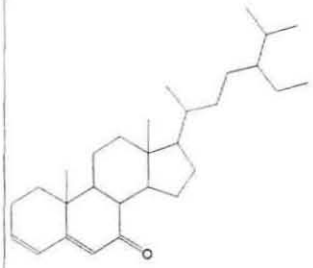


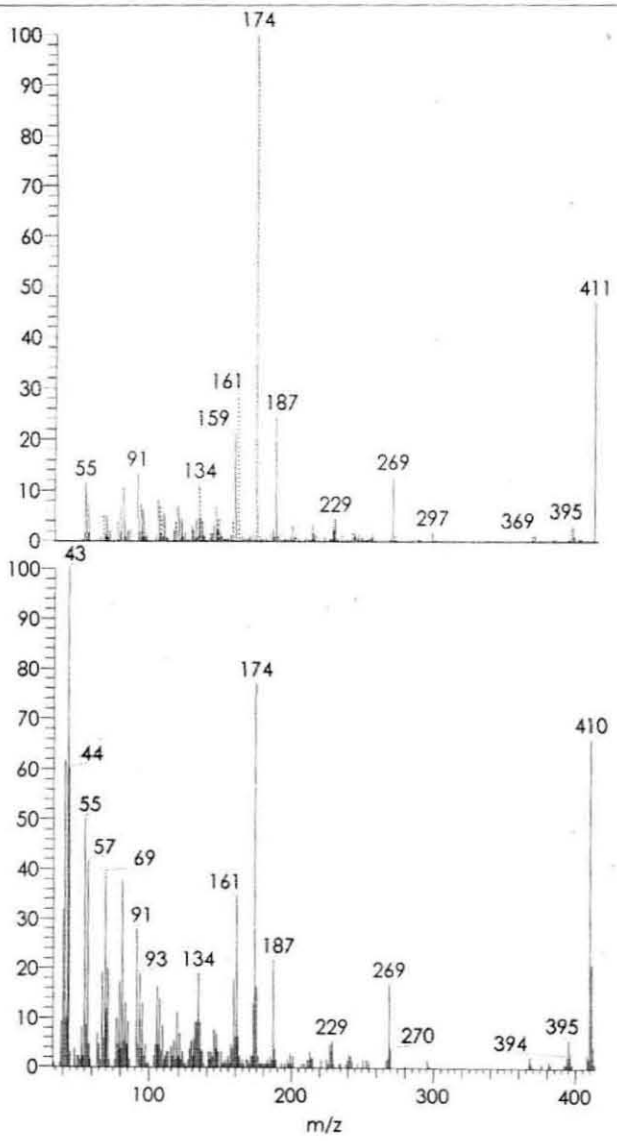

**Fig – 8 Mass spectrum of 4-3-8**

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 640 | 653 | Stigmasta-3,5- | |
| 2 | 582 | 678 | Cholesta-3,5-d | |
| 3 | 569 | 681 | Cholesta-3,5-d | |
| 4 | 561 | 666 | Cholesta-3,5-d | |
| 5 | 501 | 587 | Retinal, 9-cis- | |
| 6 | 480 | 482 | 3-(1,5-Dimethy | |
| 7 | 475 | 585 | Androst-5-en-7 | |
| 8 | 471 | 588 | Androsta-3,5-d | |
| 9 | 467 | 554 | 2-(7-Hydroxyn | |
| 10 | 455 | 459 | Lanost-8-en-3- | |
| 11 | 453 | 562 | Oxymetholone | |
| 12 | 452 | 538 | Androstan-3-c | |
| 13 | 450 | 451 | 9,19-Cyclochic | |
| 14 | 447 | 516 | Cholest-5-en-3 | |
| 15 | 442 | 544 | Cholest-5-ene | |
| 16 | 438 | 507 | Androst-5-en-3 | |
| 17 | 434 | 434 | Olean-13(18)- | |
| 18 | 430 | 488 | Pregn-4-en-3-c | |

Stigmasta-3,5-dien-7-one

β-Saccharostenone






2-4#4307 RT: 34.03 AV: 1 NL: 1.05E6 T: {0,0} + c EI det=350.00 Full ms [ 50.00-550.00]




SI 640, RSI 653, MAINLIB, Entry# 9310, CAS# 2034-72-2; Stigmasta-3,5-dien-7-one

Raw data - Library entry

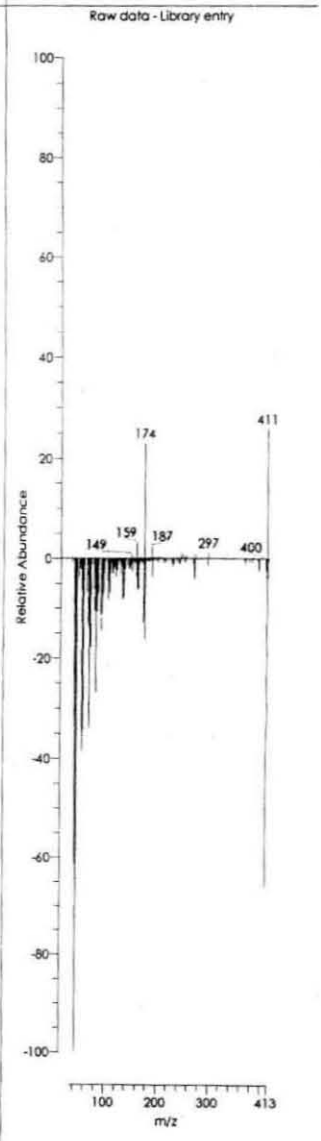



**Fig – 9 Mass spectrum of 4-3-10**

**References**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2. RJ Andersen & RJ Stonard, *Can. J. Chem.*, 1979, **57**, 2325.
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7. JA Palermo, MF R Brasco, E Cabezas, V Balzaretti & AM Seldes, *J. Nat. Prod.*, 1998, **61**, 488.
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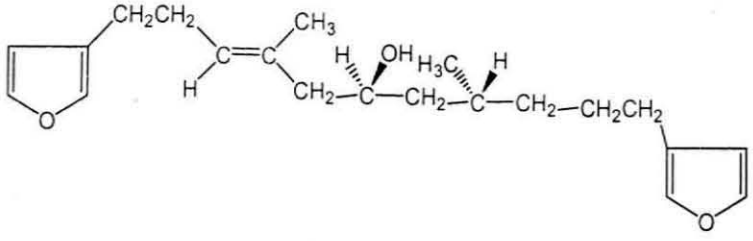
Chapter 5

# Chemical investigation of the sponge *Hippospongia* spp.

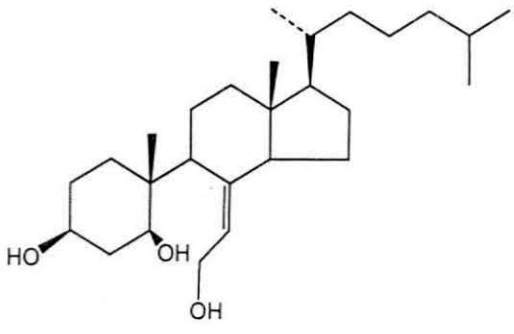
**Compounds of sponge genus *Hippospongia* spp.**

The sponge genus, *Hippospongia* spp. has been widely studied and a good parade of novel compounds have been isolated. A C-21 furanoterpene, furospongin-1 (**1**)[1] was isolated as major component from *H. communis* by Fattorusso *et al.* from ether soluble portion of the methanolic extract. Other minor compounds like furospongin-2, isofurospongin-2, dihydrofurospongin-2 and tetrahydrofurospongin-2 were subsequently isolated from the same sponge.[2]

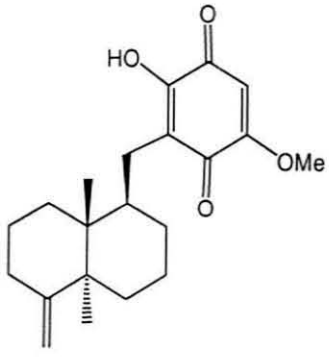


**1**

A novel trihydroxylated 5,6-secosterol (**2**)[3] was isolated from *H. communis* by Sica *et al.* from the acetone soluble portion of chloroform-methanol extract.

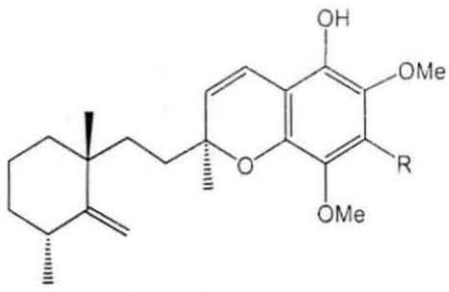


**2**

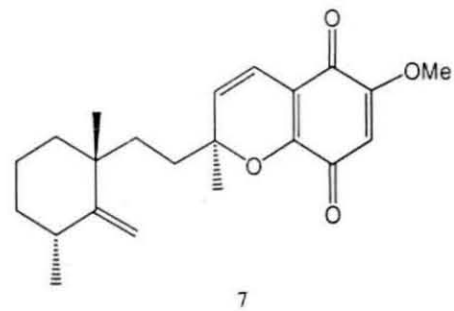


**3**

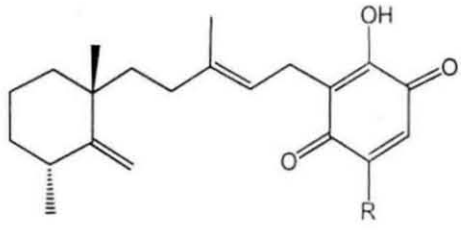
Scheuer *et al.* isolated a sesquiterpenoid quinone, ilimaquinone (**3**)[4] from the ether soluble portion of aqueous methanol-acetone extract of *H. metachromia* and it had mild antimicrobial activity. Kobayashi *et al.* isolated a novel antineoplastic sesquiterpenoid quinone and a chromenol named metachromins A (**4**) and B (**5**) from the ethyl acetate soluble fraction of methanolic extract of *H. metachromia*[5] along with a known quinone compound, isospongiaquinone.

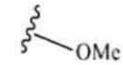


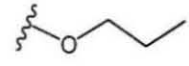
**5**, R = H
**6**, R = OMe

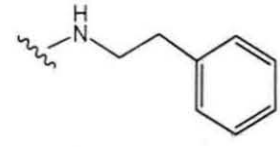


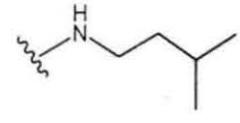
7



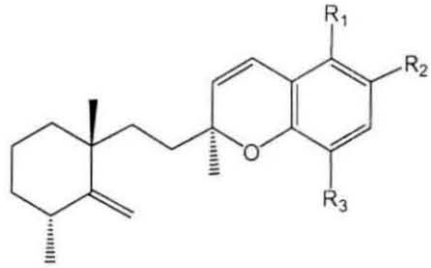
**4** R = 

**8** R = 

**9** R = 

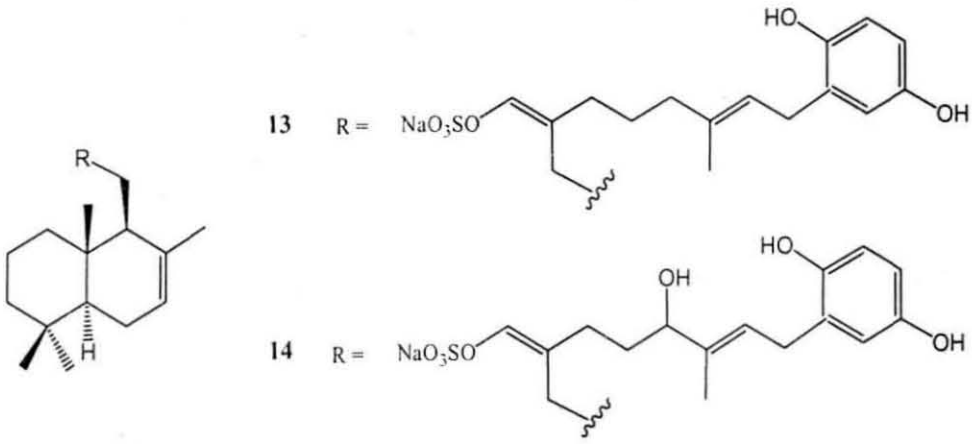
**10** R = 

Other minor compounds like metachromins C[6] and D-H (**6-10**)[7] were isolated from the same ethyl acetate soluble fraction subsequently. Shen *et al.* have recently isolated two new sesquiterpene hydroquinones, hippochromins A (**11**) and B (**12**)[8] along with known compounds from the chloroform soluble portion of the acetone extract of the same sponge species and found that compounds **4** and **5**, hippochromin A diacetate and monoacetate of **5** are potent cytotoxic against human colon (COLO-205) and nasopharyngeal (KB) tumor cells.

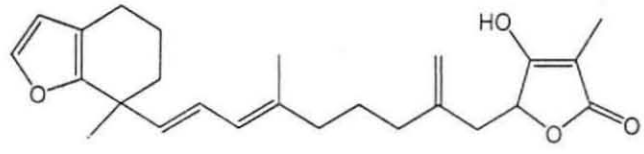


**11** $R_1 = R_2 = OH$, $R_3 = OMe$
**12** $R_1 = R_3 = OH$, $R_2 = OMe$

Higa *et al.* have isolated new sesterterpene sulphates, hipposulphates A (**13**) and B (**14**)[9] from Okinawan *H. metachromia* from the chloroform soluble portion of the acetone extract.

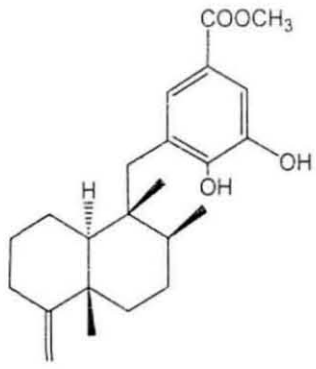



Apart from these two species, other types of compounds have also been isolated from undescribed species of this genus. A furanosesterterpene, hippospongin (**15**)[1c] possessing antispasmodic activity was isolated from Okinawan *Hippospongia* spp. from the chloroform soluble portion of methanol extract.

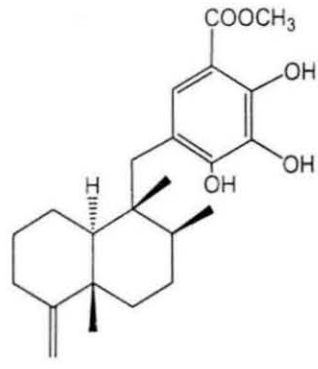



**15**

Antimicrobial sesquiterpenoids, dictyoceratin - A (**16**) and - B (**17**) along with furospinulosin-1 (**18**) and ilimaquinone (**3**) from hexane soluble portion of the ethanolic extract of the same Okinawan sponge genus[10] were obtained.




16

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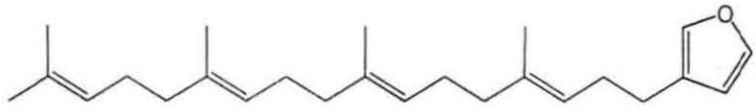
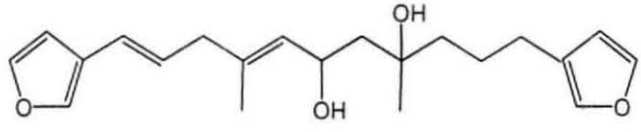



18

Two new furanoterpenes like (**1**), untenospongins - A (**19**) and - B (**20**) were obtained from the ethyl acetate soluble portion of methanol-toluene (3:1) extract of Okinawan *Hippospongia* spp[11], and they were found to be potent coronary vasodilating components.

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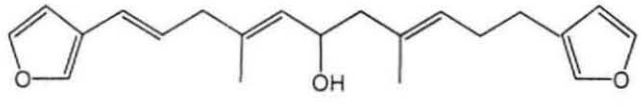
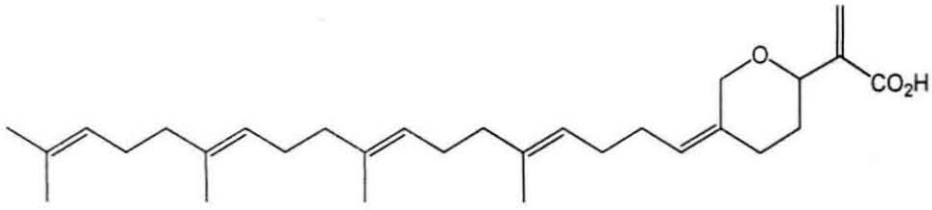



20

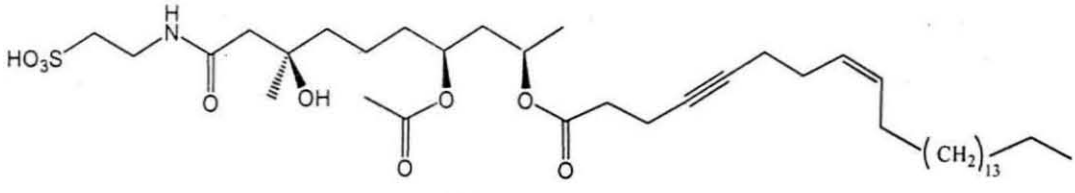
The acetone-chloroform soluble fraction of methanol extract of *Hippospongia* spp. afforded hippospongic acid-A (**21**)[12] on further fractionation using hexane followed

by silica gel column and HPLC separation methods. This compound was found to potently inhibited gastrulation of starfish embryos.



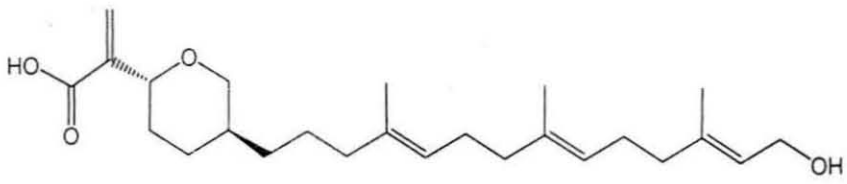

**21**

Taurine-containing acetylenic lipid, taurospongin-A (**22**), which inhibits HIV reverse transcriptase has been isolated from the *n*-butanol soluble fraction of methanol extract of the same sponge genus.[13]

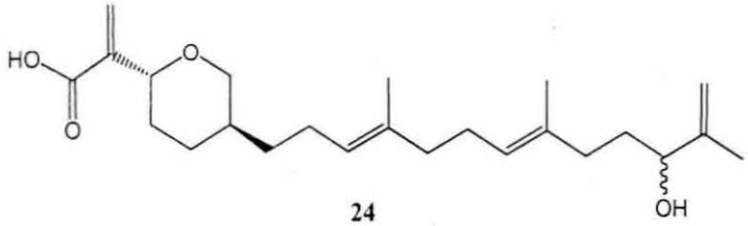



**22**

Recently novel terpenoids with RCE protease inhibitory activity, barangcadoic acid-A (**23**) as major and rhopaloic acids-D (**24**) to -G as minor compounds have been obtained from the ethyl acetate soluble portion of ethanol extract of Indonesian sponge *Hippospongia* spp.[14]

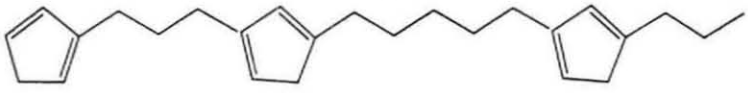



**23**

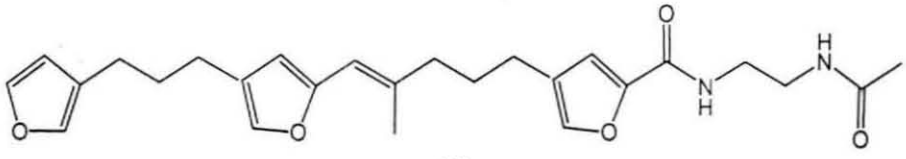



**24**

Six new furanoterpenes of varying chain length from $C_{22}$ to $C_{25}$, hipposponginsA (**25**)-F (**26**) were isolated from dichloromethane solubles of ethanol extract of Australian *Hippospongia* spp.[15]



**25**




**26**

**Chemical Analysis of Sponge *Hippospongia* spp.**

| | | |
|---|---|---|
| Phylum | - | Porifera |
| Class | - | Demospongia |
| Sub class | - | Ceractinomorpha |
| Order | - | Dictyoceratida |
| Family | - | Spongiidae (Gray 1867) |
| Genus | - | *Hippospongia* spp. (Schulze 1879) |

**Fig - 1**

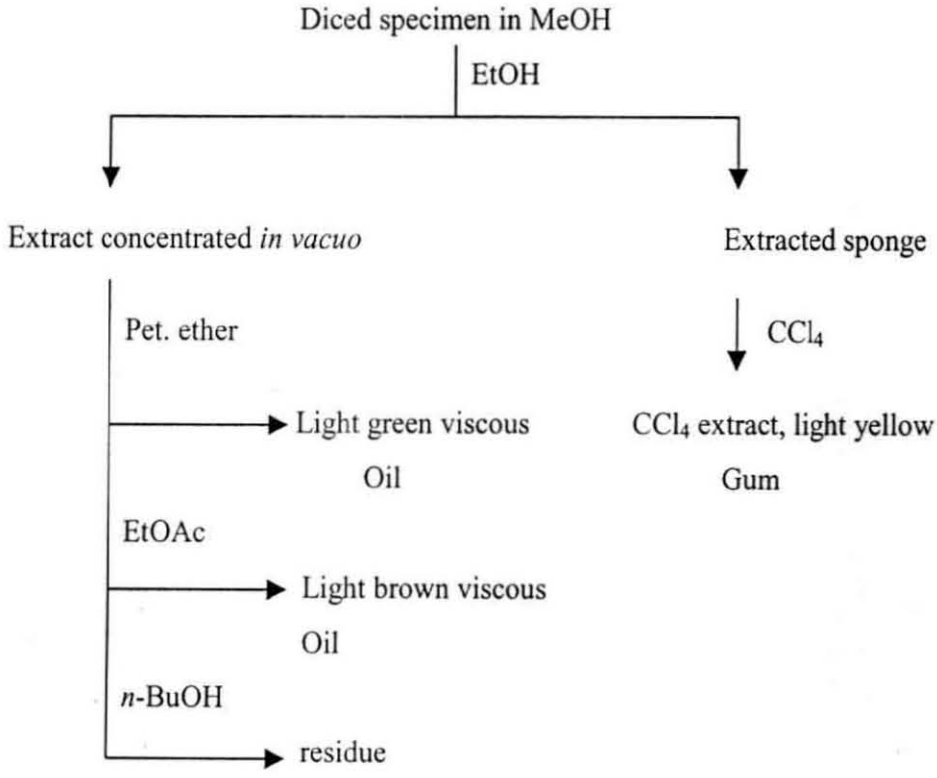
Extraction of sponge, *Hippospongia* spp. (**Fig-1**)

The initial alcoholic sponge extract (~1600 ml) was decanted and the sponge (2 kg) was extracted with ethyl alcohol (500 ml, 2 times). The combined alcoholic extracts were filtered and concentrated both under air and *in vacuo*. The concentrated extract was fractionated successively with pet. ether, ethyl acetate and *n*-butanol and the residues were recovered from each of them to get the respective fractions. The ethyl acetate and *n*-butanol extracts do not contain any interesting organic compound (**Scheme-1**).

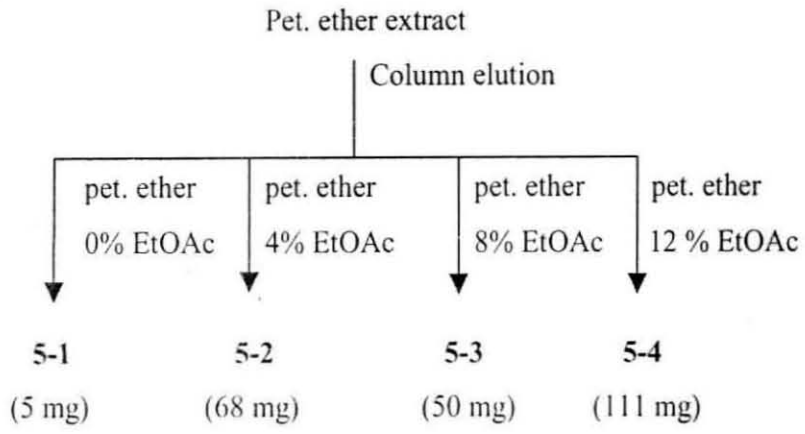
The extracted sponge was directly treated with carbon tetrachloride to get a residue in order to ascertain the nature of compounds present in the sponge extracted directly and by the collective alcoholic extraction. Examination of crude fractions by GC showed that this fraction has the same components as found in the pet. ether extract of the original alcoholic extract.

The pet. ether extracts was then subjected to column chromatography using silica eluting with increasing concentration of ethyl acetate in pet. ether from 0 -12 % (**Scheme 2**). Four fractions, fractions **5-1, 5-2, 5-3** and **5-4** have been obtained during this process. These fractions were then collected by monitoring by *tlc* of the eluates from the column were recovered and they were analyzed by GC-MS.

**Extraction and Fractionation of *Cervicornia* sp.**




**Scheme 1**




**scheme 2**

Analysis of fraction **5-1 (Table - 1)**

There are 9 compounds (**5-1-1** to **5-1-9**) and they were identified as simple straight chain hydrocarbons from the chain length of $C_{14}$ to $C_{29}$. The molecular ion peaks appear mostly as $M^+$/M+1. The base peak is at 57 for all the compounds accounting for the fragment unit of $[C_4H_9]^+$ followed by 71, 85, 99, 113 *m/e* values with a mass difference of 14 accountable for $CH_2$, showing the presence of characteristic straight chain alkyl unit. Compounds with chain length above 20 carbons [**5-1-6 (Fig - 2)** to **5-1-8 (Fig – 3)** and **5-1-9**] have more contribution between 10% and 20% in this fraction. Other compounds, **5-1-1** to **5-1-5** contribute only in traces. Higher homologues have higher RT values. The data of the compounds present in this fraction have been given in **Table - 1**.

Analysis of fraction **5-2 (Table - 2)**

This fraction has nine compounds [**5-2-1 (Fig – 4)** to **5-2-6 (Fig – 6), 5-2-7** to **5-2-9**] identified mostly as the methyl esters of long chain fatty acids of both saturated and unsaturated nature except for compound **5-2-1**, which is *n*-hexanal. Saturated esters have strong M+1 except compound **5-2-3** and have their base peak at *m/e* 74 accountable for $[CH_2=C(OH)OMe]^+$arising out of McLafferty rearrangement. Other fragments are of typical straight chain hydrocarbons with regular difference of 14 mass unit.

**Table – 1:** Analysis of fraction **5-1**

| Comp No. | RT | Peak area %age | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|---|
| **5-1-1** | 4.84 | 1.47 | $C_{14}H_{30}$ | 198 |  *n*-tetradecane | 198 | 57, 71, 85, 55, 99, 112 |
| **5-1-2** | 7.21 | 7.06 | $C_{16}H_{34}$ | 226 |  *n*-hexadecane | 226 | 57, 71, 85, 55, 99, 113, 127 |
| **5-1-3** | 8.34 | 2.83 | $C_{15}H_{32}$ | 212 |  *n*-pentadecane | 210 | 57, 71, 85, 55, 99, 113 |
| **5-1-4** | 9.44 | 7.62 | $C_{18}H_{38}$ | 254 |  *n*-octadecane | 254 | 57, 71, 85, 99, 113 |
| **5-1-5** | 11.50 | 1.91 | $C_{20}H_{42}$ | 282 |  *n*-eicosane | 282 | 57, 71, 85, 99, 113 |
| **5-1-6** | 17.59 | 17.75 | $C_{27}H_{56}$ | 380 |  *n*-heptacosane | 323 | 57, 71, 85, 99, 113, 127 |
| **5-1-7** | 18.61 | 14.99 | $C_{28}H_{58}$ | 394 |  *n*-octacosane | 395 | 57, 71, 85, 99, 113, 127, 155 |
| **5-1-8** | 19.88 | 19.60 | $C_{29}H_{60}$ | 408 |  *n*-nonacosane | 409 | 57, 71, 85, 99, 113, 127, 155 |
| **5-1-9** | 23.57 | 17.87 | $C_{25}H_{52}$ | 352 |  *n*-pentacosane | 351 | 57, 71, 85, 99, 113, 127, 141, 155 |

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 752 | 805 | Heptacosane | |
| 2 | 750 | 751 | Docosane | |
| 3 | 741 | 753 | Heptacosane | |
| 4 | 737 | 798 | Octadecane | |
| 5 | 737 | 799 | Eicosane | |
| 6 | 737 | 744 | Tricosane | |
| 7 | 736 | 746 | Heneicosane | |
| 8 | 735 | 776 | Heneicosane | |
| 9 | 734 | 746 | Eicosane | |
| 10 | 734 | 751 | Docosane | |
| 11 | 732 | 804 | Octadecane | |
| 12 | 731 | 794 | Heptadecane | |
| 13 | 731 | 794 | Heptadecane | |
| 14 | 730 | 808 | Eicosane | |
| 15 | 730 | 751 | Pentacosane | |
| 16 | 728 | 733 | Tricosane | |
| 17 | 728 | 743 | Octadecane | |
| 18 | 727 | 813 | Pentadecane, 2-methyl- | |

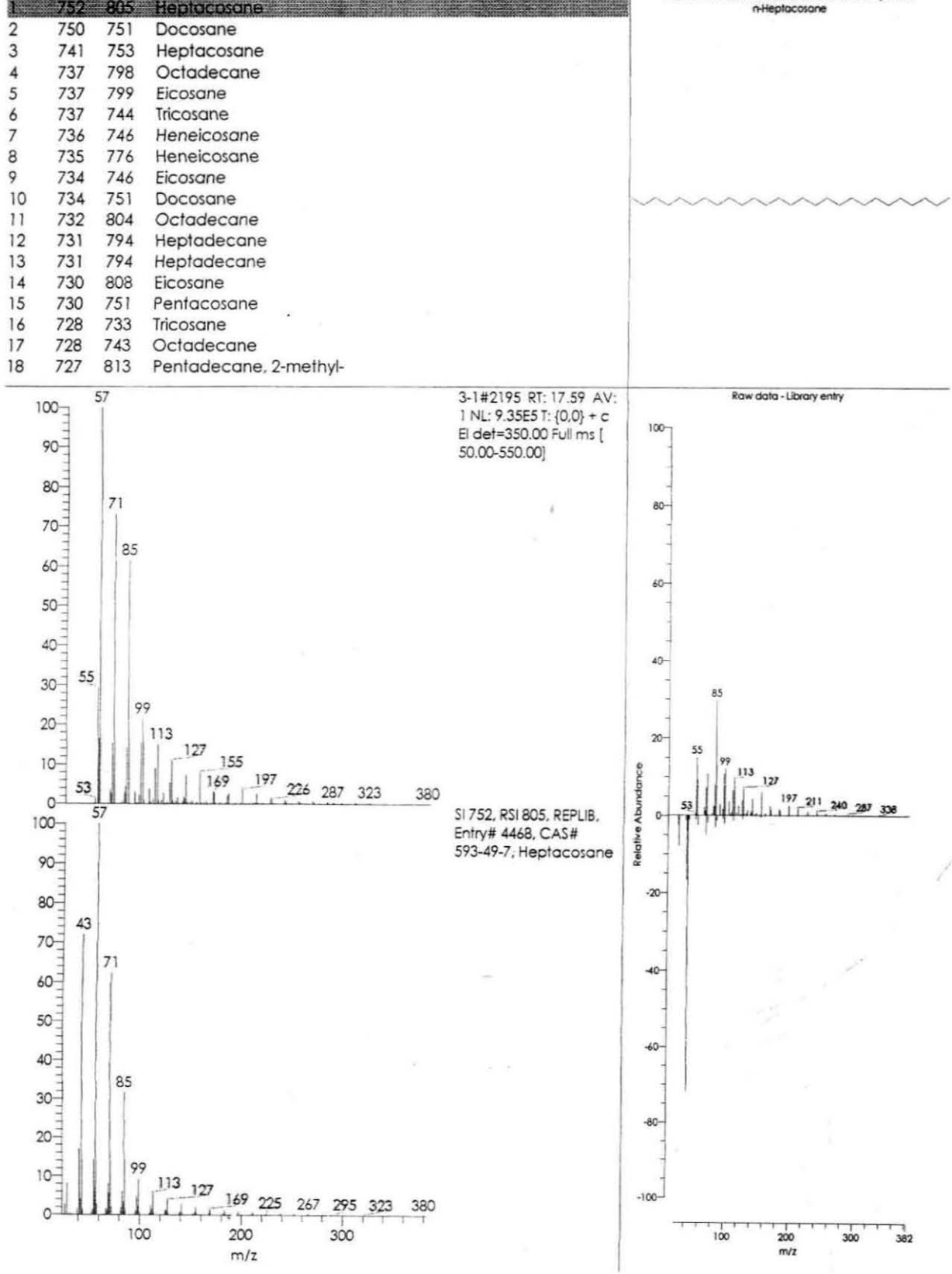



**Fig – 2 Mass spectrum of 5-1-6**

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 3 | 773 | 818 | Hexacosane | |
| 4 | 771 | 782 | Docosane | |
| 5 | 768 | 768 | Nonacosane | |
| 6 | 768 | 776 | Tricosane | |
| 7 | 768 | 783 | Pentacosane | |
| 8 | 767 | 813 | Eicosane | |
| 9 | 767 | 769 | Hexacosane | |
| 10 | 764 | 765 | Octacosane | |
| 11 | 762 | 770 | Nonacosane | |
| 12 | 760 | 769 | Tetratriacontane | |
| 13 | 759 | 779 | Hexacosane | |
| 14 | 759 | 768 | Octacosane | |
| 15 | 758 | 759 | Nonacosane | |
| 16 | 754 | 774 | Octacosane | |
| 17 | 754 | 782 | Pentacosane | |
| 18 | 752 | 764 | Pentatriacontane | |
| 19 | 750 | 766 | Eicosane | |
| 20 | 748 | 750 | Tetratetracontane | |

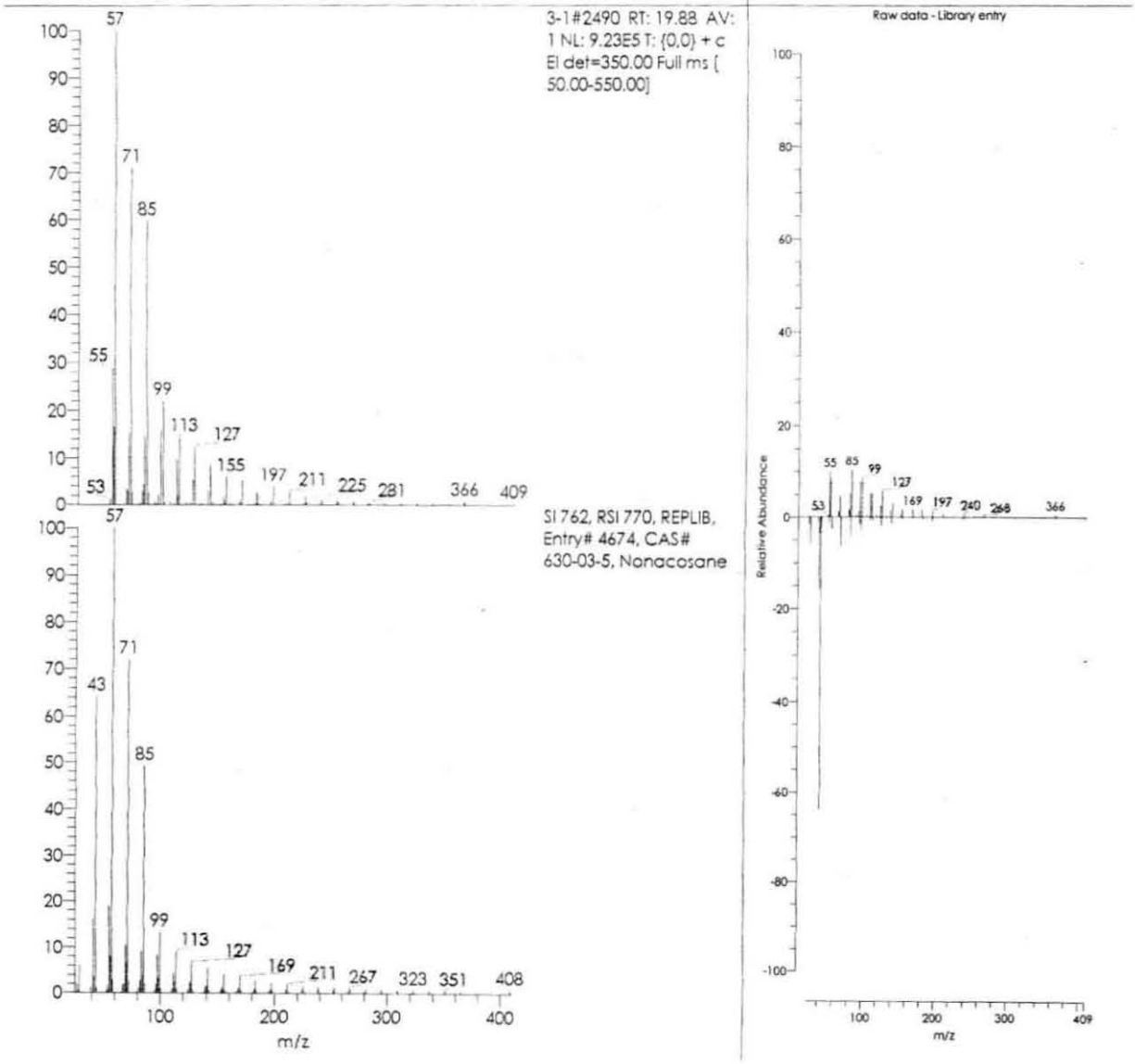
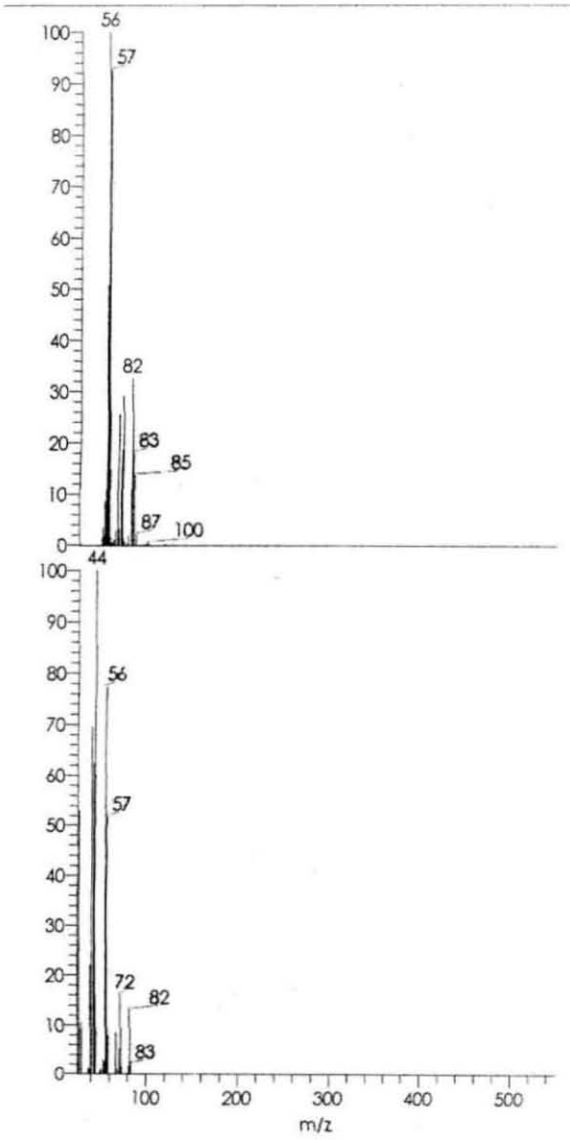



**Fig – 3 Mass spectrum of 5-1-8**

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 827 | 869 | Hexanal | |
| 2 | 809 | 817 | Hexanal | |
| 3 | 790 | 792 | Hexanal | |
| 4 | 776 | 789 | Cyclopentanol, 2-methyl-, trans- | |
| 5 | 771 | 782 | Cyclopentanol, 3-methyl- | |
| 6 | 765 | 882 | Hexanal | |
| 7 | 758 | 762 | 2-Hexen-1-ol, (E)- | |
| 8 | 757 | 762 | 2-Hexen-1-ol, (E)- | |
| 9 | 753 | 806 | Cyclohexane, (1,1-dimethylethyl)- | |
| 10 | 750 | 765 | 2-Hexen-1-ol, (E)- | |
| 11 | 750 | 768 | Cyclopentanol, 2-methyl-, cis- | |
| 12 | 748 | 759 | Cyclopentanol, 2-methyl- | |
| 13 | 747 | 754 | Cyclopentanol, 3-methyl- | |
| 14 | 746 | 758 | Cyclopentanol, 2-methyl- | |
| 15 | 744 | 778 | 2-Hexen-1-ol, (E)- | |
| 16 | 736 | 741 | 2-Hexen-1-ol, (E)- | |
| 17 | 733 | 804 | Cyclohexane, (1,1-dimethylethyl)- | |
| 18 | 725 | 731 | Cyclohexanol | |

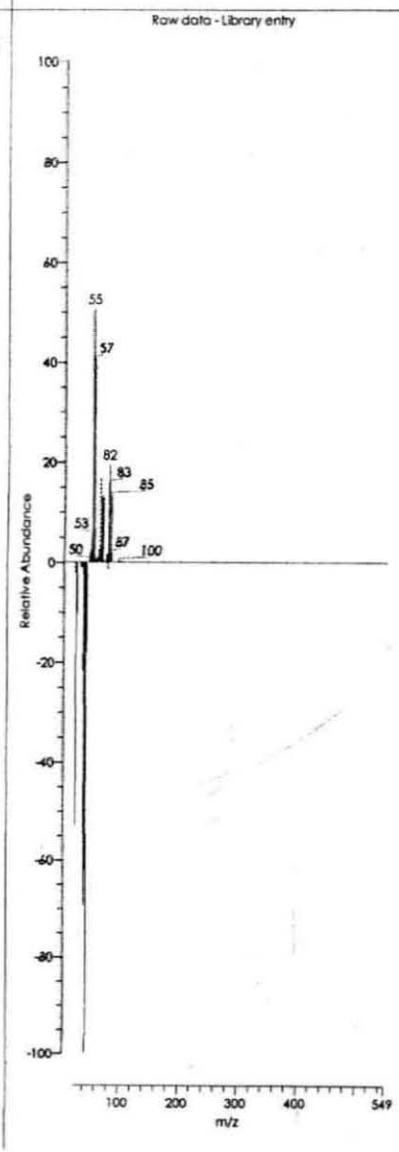
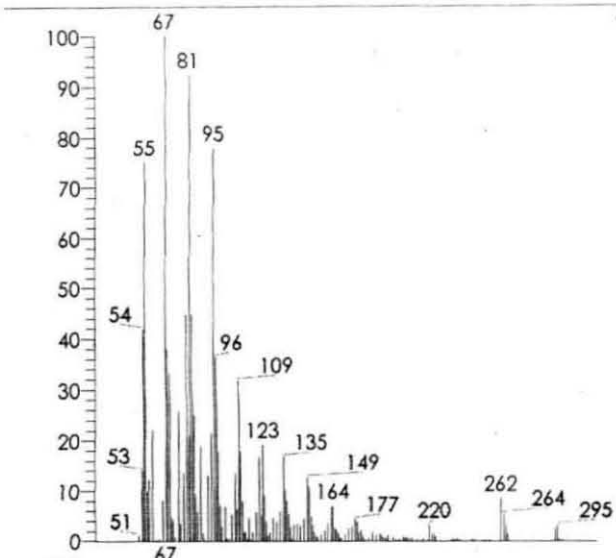




**Fig – 4 Mass spectrum of 5-2-1**

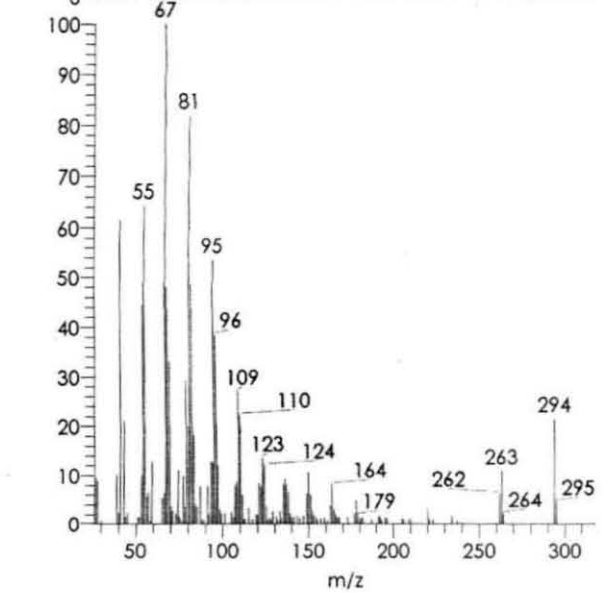
| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 820 | 841 | 9,12-Octadec | |
| 2 | 817 | 830 | 9,12-Octadec | |
| 3 | 808 | 834 | 9,12-Octadec | |
| 4 | 804 | 818 | 9,12-Octadec | |
| 5 | 802 | 809 | 9,12-Octadec | |
| 6 | 793 | 796 | 9,12-Octadec | |
| 7 | 789 | 825 | 9,12-Octadec | |
| 8 | 786 | 794 | 9,12-Octadec | |
| 9 | 779 | 832 | 9,12-Octadec | |
| 10 | 778 | 789 | 8,11-Octadec | |
| 11 | 777 | 813 | 9,12-Octadec | |
| 12 | 775 | 776 | 8,11-Octadec | |
| 13 | 774 | 792 | 12,15-Octade | |
| 14 | 768 | 859 | 9,12-Octadec | |
| 15 | 768 | 794 | 14,17-Octade | |
| 16 | 767 | 797 | 9,12-Octadec | |
| 17 | 767 | 785 | 11,14-Octade | |
| 18 | 763 | 769 | 7,10-Octadec | |

9,12-Octadecadienoic acid (Z,Z)-, methyl ester

Linoleic acid, methyl ester




3-2#1548 RT: 12.55 AV: 1 NL: 1.69E6 T: {0,0} + c EI det=350.00 Full ms [ 50.00-550.00]




SI 820, RSI 841, MAINLIB, Entry# 21424, CAS# 112-63-0, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester

Raw data - Library entry

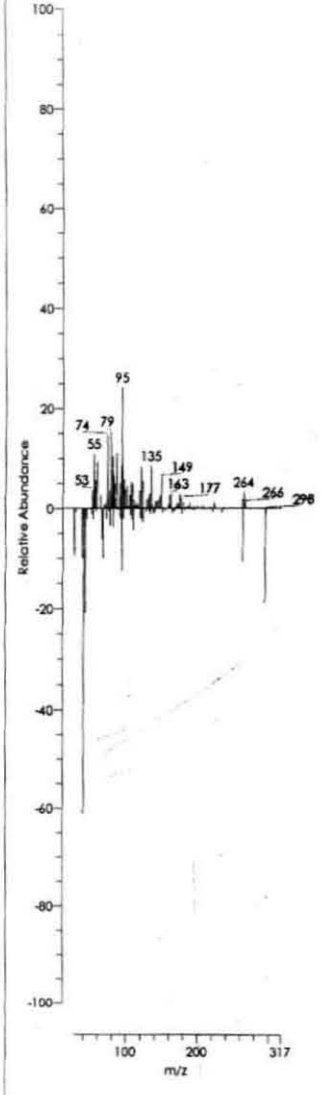



**Fig – 6 Mass spectrum of 5-2-6**

For compounds **5-2-1** and **5-2-2** (**Fig – 5**), the popular cleavage of $\beta$-$\gamma$ bond and $\gamma$-$\delta$ bond at the aldehyde end give fragments at *m/e* 55 and 72 respectively. The $\beta$-$\gamma$ bond cleavage at the ester end gives a fragment at *m/e* 87. The methyl esters detected in this fraction are even-numbered. The list of compounds with data is given in **Table - 2**.

Analysis of fraction **5-3 (Table - 3)**

Though this fraction appeared as pure component in *tlc*, it has two compounds [**5-3-1** (**Fig - 7**) and **5-3-2** (**Fig - 8**)] when analyzed by GC. Both are steroidal unsaturated hydrocarbons - cholesta-3,5-diene, **5-3-1** and stigmasta-3,5-diene, **5-3-2**. The composition is approximately equal with 44 and 56 percentages. Both exhibit prominent peaks in the mass spectrum as that of standards. The base peak is due to the terminal isopropyl unit of the molecule in both the cases. The mass spectral data of **5-3-1** and **5-3-2**, are given in **Table - 3**.

Analysis of fraction **5-4 (Table - 4)**

It has five steroidal compounds [**5-4-1** (**Fig – 9**) to **5-4-5**] and all of them are the derivatives of cholesterol. Cholesterol, **5-4-1** and $\tau$-sitosterol, **5-4-5** are the major compounds with 27 and 56 percentages. Other derivatives are below 10% of the total composition. The molecular ion of cholesterol, **5-4-1** is also the base peak. Elimination of water molecule gives the fragment at *m/e* 369. The M+1 peaks are well apparent in all the other compounds **5-4-2** to **5-4-5** and M-18 peaks are also present in them. The prominent peaks with base peak for each compound are given in **Table - 4**. The low percentage of compounds with side chain unsaturation may be indicative that they are the biosynthetic precursors for building the saturated cholesterol and its homologues.

**Table - 2:** Analysis of fraction **5-2**

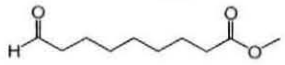
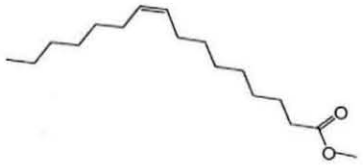
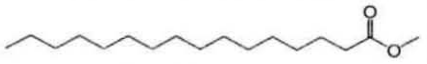
| Comp No | RT | Peak area %age | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|---|
| **5-2-1** | 0.65 | 1.00 | $C_6H_{12}O$ | 100 |  *n*-Hexanal | 100 | 56, 72, 82 |
| **5-2-2** | 5.44 | 3.06 | $C_{10}H_{18}O_3$ | 186 |  Methyl 9-oxononanoate | 168 | 74, 55, 87, 111, 143, 155 |
| **5-2-3** | 8.71 | 6.13 | $C_{15}H_{30}O_2$ | 242 |  Methyl tetradecanoate | 242 | 74, 87, 55, 143, 69, 199 |
| **5-2-4** | 10.65 | 3.99 | $C_{17}H_{32}O_2$ | 268 |  Methyl 9(Z)-hexadecenoate | 269 | 55, 74, 87, 69, 96, 105, 152, 194, 236 |
| **5-2-5** | 10.96 | 46.57 | $C_{17}H_{34}O_2$ | 270 |  Methyl hexadecanoate | 271 | 74, 87, 42, 55, 143, 227 |

**Table – 2 contd...**

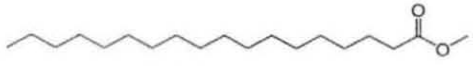
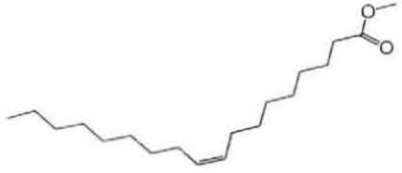
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **5-2-6** | 12.55 | 22.41 | $C_{19}H_{34}O_2$ | 294 |  Methyl 9,12-octadecadienoate | 295 | 67, 81, 95, 55, 109, 123, 135, 262 |
| **5-2-7** | 12.83 | 5.69 | $C_{19}H_{38}O_2$ | 298 |  Methyl octadecanoate | 299 | 74, 87, 55, 143, 97, 255 |
| **5-2-8** | 14.36 | 4.31 | $C_{19}H_{36}O_2$ | 296 |  Methyl 9(Z)-octadecenoate | 294 | 55, 42, 74, 83, 96, 110, 264, 222 |
| **5-2-9** | 14.61 | 6.83 | $C_{21}H_{42}O_2$ | 326 |  Methyl eicosanoate | 269 | 55, 74, 87, 69, 96, 105, 152, 194, 236 |

Table - 3: Analysis of fraction **5-3**

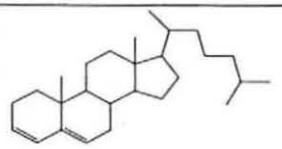
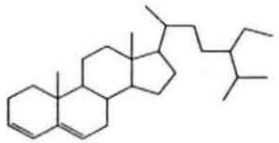
| Comp No. | RT | Peak area %age | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|---|
| **5-3-1** | 23.87 | 43.52 | $C_{27}H_{44}$ | 368 |  Cholesta-3,5-diene | 370 | 42, 145, 105, 81, 159, 255 |
| **5-3-2** | 29.52 | 56.48 | $C_{29}H_{48}$ | 396 |  Stigmasta-3,5-diene | 398 | 42, 145, 159, 382 |

Table - 4: Analysis of fraction **5-4**

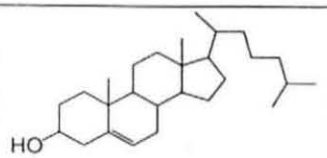
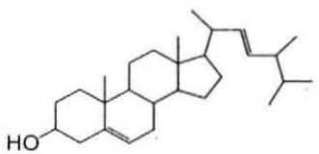
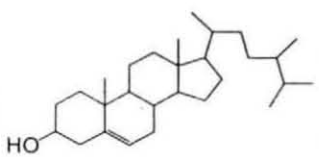
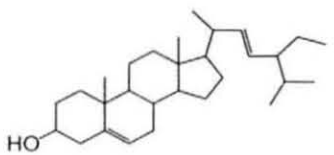
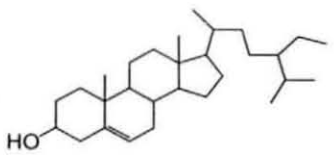
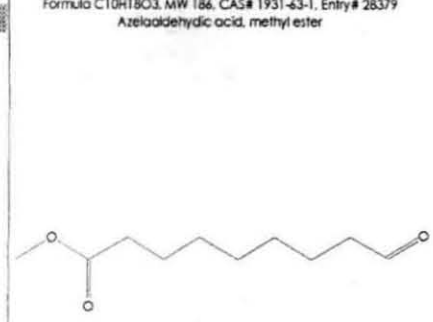
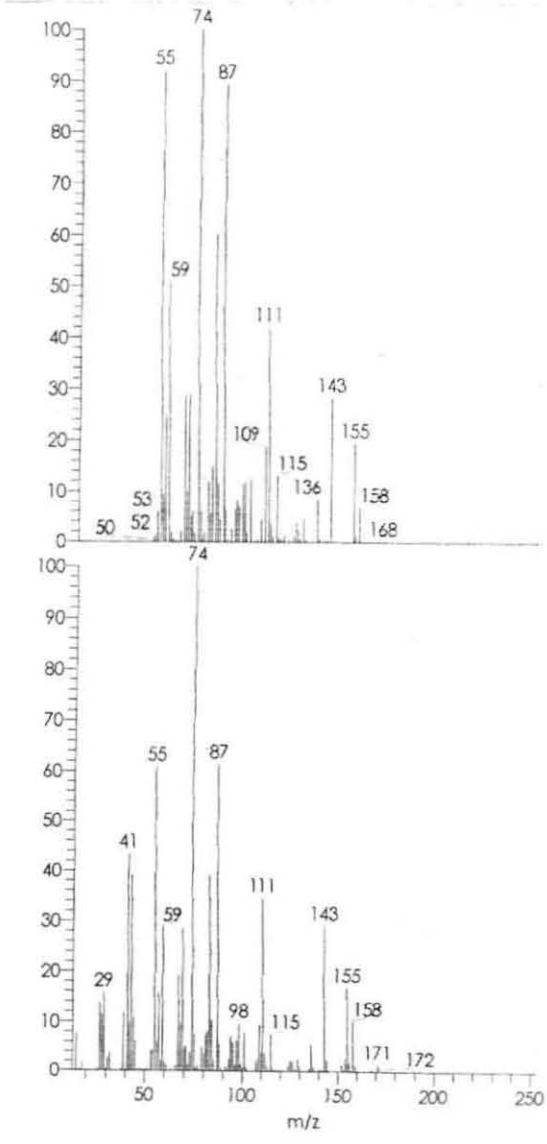
| Comp No. | RT | Peak area %age | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|---|
| **5-4-1** | 24.21 | 27.32 | $C_{27}H_{46}O$ | 386 |  Cholesterol | 387 | 387, 95, 145, 81, 107, 55, 369 |
| **5-4-2** | 25.07 | 4.05 | $C_{28}H_{46}O$ | 398 |  Ergosta-5,22-dien-3-ol | 400 | 55, 69, 159, 255, 145, 133, 81, 91, 107 |
| **5-4-3** | 26.75 | 8.80 | $C_{28}H_{48}O$ | 400 |  Ergosta-5-en-3-ol (Campesterol) | 403 | 145, 105, 95, 159, 55, 81, 213, 255, 316 |

**Table – 4 contd...**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **5-4-4** | 27.74 | 4.26 | $C_{28}H_{48}O$ | 412 |  Stigmasta-5,22-dien-3-ol (Stigmasterol) | 413 | 55, 83, 69, 105, 133, 159, 145, 213, 255 |
| **5-4-5** | 30.36 | 5.57 | $C_{29}H_{50}O$ | 414 |  Stigmasta-5-en-3-ol (*i*-Sitosterol) | 415 | 107, 145, 95, 120, 81, 65, 135, 163, 173 |

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 829 | 829 | Nonanoic acid, 9-oxo-, m | |
| 2 | 685 | 745 | Decanoic acid, methyl es | |
| 3 | 673 | 744 | Decanoic acid, methyl es | |
| 4 | 662 | 684 | Decanoic acid, methyl es | |
| 5 | 660 | 668 | Decanoic acid, methyl es | |
| 6 | 648 | 717 | Dodecanoic acid, methyl | |
| 7 | 646 | 683 | Dodecanoic acid, methyl | |
| 8 | 643 | 684 | Octanoic acid, methyl est | |
| 9 | 640 | 953 | Nonanoic acid, 9-oxo-, m | |
| 10 | 638 | 695 | Cyclopentaneundecano | |
| 11 | 635 | 670 | Dodecanoic acid, methyl | |
| 12 | 635 | 635 | 9-Hydroxy-decanoic acid | |
| 13 | 634 | 696 | Pentadecanoic acid, met | |
| 14 | 633 | 673 | Tridecanoic acid, methyl e | |
| 15 | 630 | 671 | Nonanoic acid, methyl es | |
| 16 | 629 | 638 | Methyl tetradecanoate | |
| 17 | 626 | 723 | Undecanoic acid, 2-meth | |
| 18 | 625 | 658 | Undecanoic acid, methyl | |



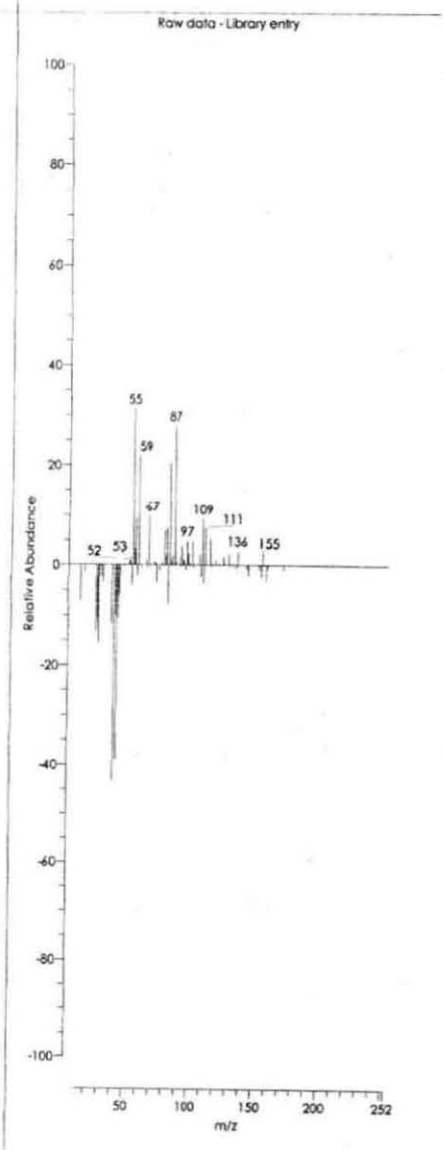
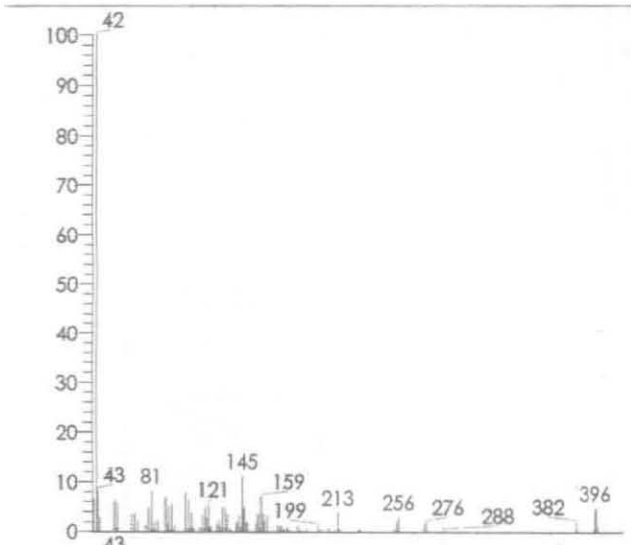
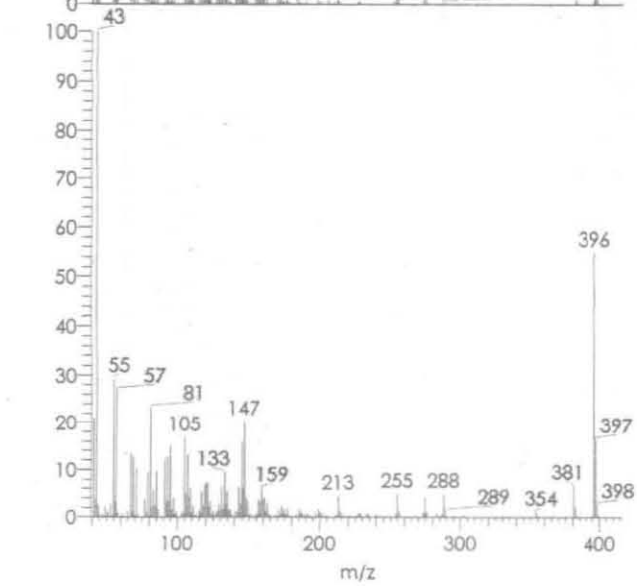




**Fig – 5 Mass spectrum of 5-2-2**

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 666 | 667 | β-Sitosterol ac | |
| 2 | 649 | 653 | Stigmastan-3, | |
| 3 | 639 | 696 | β-Sitosterol ac | |
| 4 | 637 | 676 | τ-Sitosterol | |
| 5 | 632 | 639 | Stigmastan-3-c | |
| 6 | 621 | 698 | Cholest-5-en-3 | |
| 7 | 620 | 729 | Cholest-5-ene | |
| 8 | 614 | 749 | Dihydrotachys | |
| 9 | 603 | 691 | Cholest-5-en-3 | |
| 10 | 599 | 600 | Stigmast-5-en- | |
| 11 | 597 | 654 | Campesterol | |
| 12 | 592 | 649 | Cholest-5-en-3 | |
| 13 | 590 | 596 | τ-Sitosterol | |
| 14 | 585 | 652 | Cholest-5-en-3 | |
| 15 | 584 | 587 | Sitosterol acet | |
| 16 | 582 | 617 | Methyl (25rs)-3 | |
| 17 | 579 | 645 | Retinol | |
| 18 | 571 | 685 | 24-Norchol-22 | |


3-3a#3368 RT: 29.52
AV: 1 NL: 2.79E5 T: {0.0} + c EI det=350.00 Full ms [ 40.00-600.00]




SI 649, RSI 653, MAINLIB, Entry# 10351, CAS# NA, Stigmastan-3,5-dien

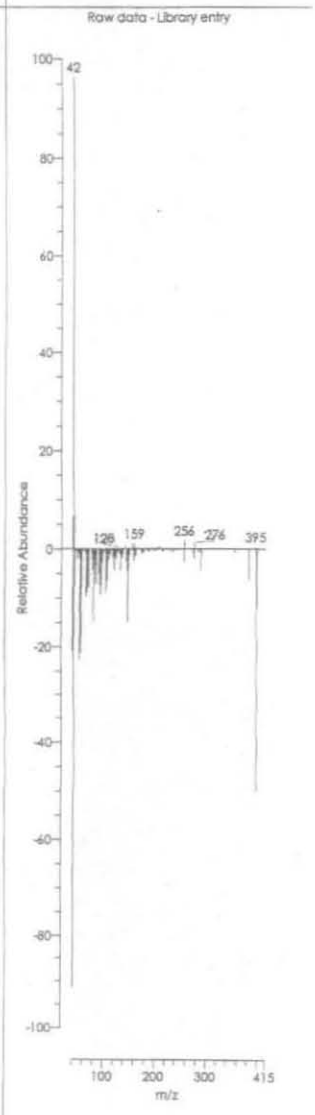
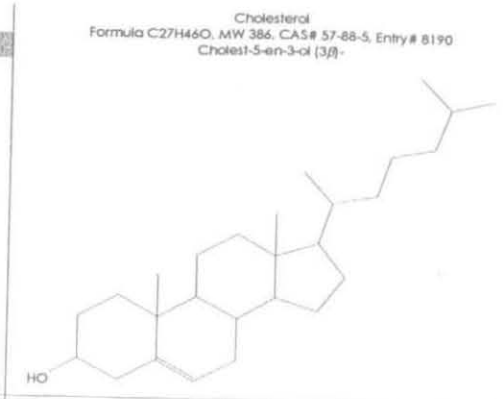
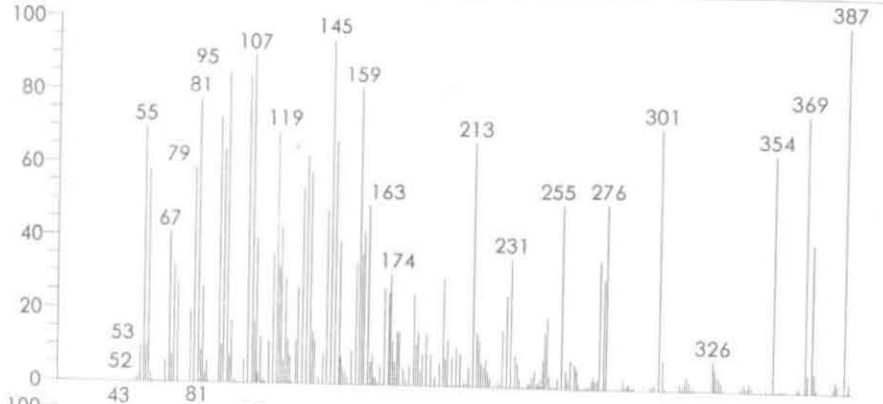


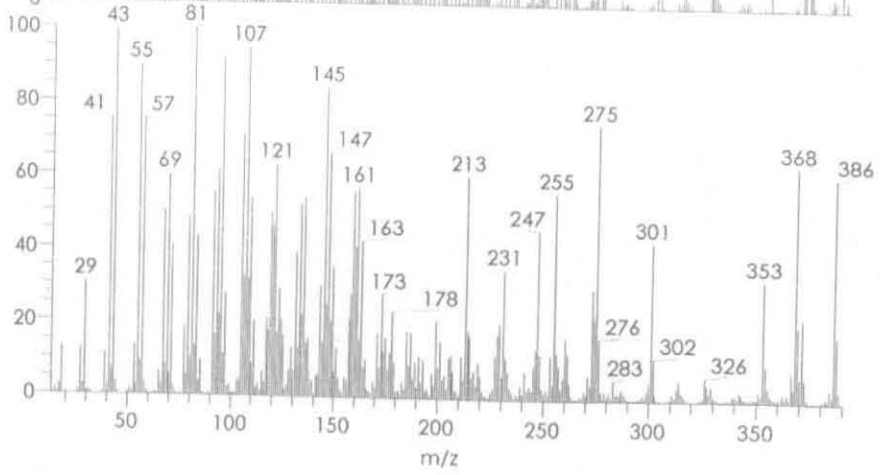

Fig – 8 Mass spectrum of 5-3-2

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 687 | 687 | Cholesterol | |
| 2 | 683 | 717 | Cholesterol | |
| 3 | 683 | 717 | Cholesterol | |
| 4 | 675 | 676 | Cholesterol | |
| 5 | 672 | 674 | Cholesterol | |
| 6 | 631 | 635 | 17-(1,5-Dimethylhe | |
| 7 | 612 | 614 | Cholestane-3,5-di( | |
| 8 | 611 | 622 | 26-Nor-5-cholester | |
| 9 | 595 | 623 | Cholesteryl benzo( | |
| 10 | 593 | 641 | Cholesta-3,5-diene | |
| 11 | 590 | 689 | Cholest-5-en-3-ol ( | |
| 12 | 587 | 628 | Cholest-7-en-3-ol, | |
| 13 | 576 | 577 | Cholest-5-en-3-ol ( | |




3-4#3046 RT: 24.21 AV: 1 NL: 3.18E5 T: {0,0} + c EI det=350.00 Full ms [ 50.00-550.00]




SI 687, RSI 687, REPLIB, Entry# 8190, CAS# 57-88-5, Cholesterol

Raw data - Library entry

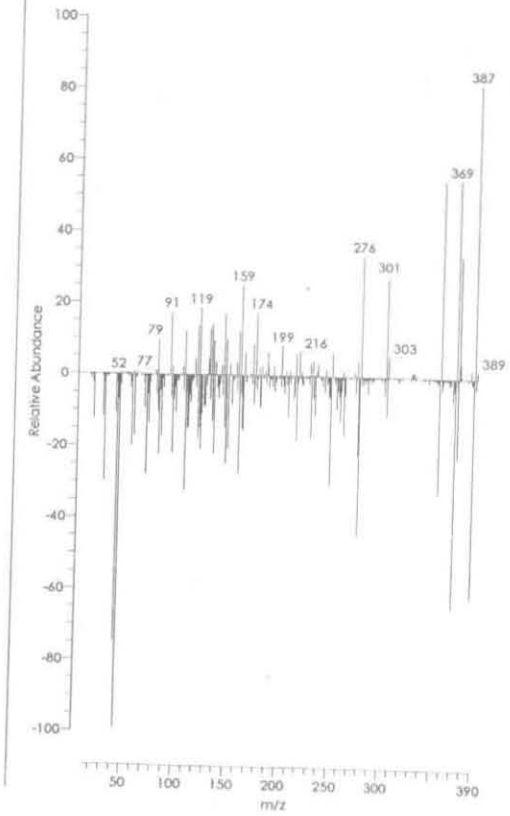



**Fig – 9 Mass spectrum of 5-4-1**

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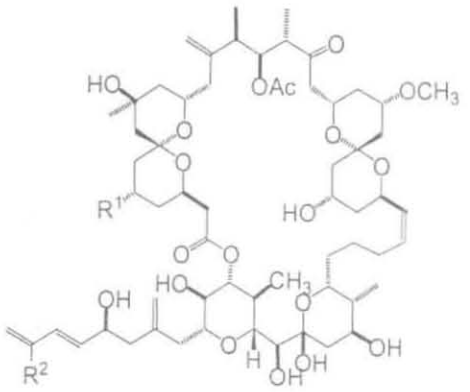
Chapter 6

# Chemical investigation of the sponge *Hyrtios* spp.

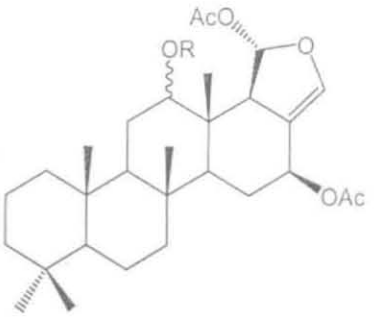
**Compounds of sponge genus *Hyrtios* spp.**

The sponge genus *Hyrtios* spp. is a good storehouse of variety of compounds with potential bioactivity as has been proved by repeated reports. Kobayashi *et al.* isolated a new class of extremely cytotoxic macrolides called altohyrtins A (**1**), B (**2**), C (**3**) and 5-deacetylaltohyrtin A (**4**)[1] which were present in trace amount in the acetone soluble portion of *Hyrtios altum*.

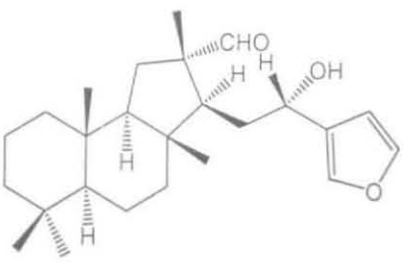


| | $R^1$ | $R^2$ |
|---|---|---|
| 1, | OAc | Cl |
| 2, | OAc | Br |
| 3, | OAc | H |
| 4, | OH | Cl |

Several scalarane[2a,2b] type sesterterpenoids, heteronemin (**5a**)[3a], 12-*epi*-heteronemin (**5b**)[3b] and 12-*epi*-heteronemin acetate (**5c**)[2c] were isolated from *H. erecta*. A tricyclic sesterterpenoid, hyrtiosal (**6**) having antitumor property, was isolated from the ethyl acetate soluble portion of methanol extract of Okinawan *H. erecta*.[4]

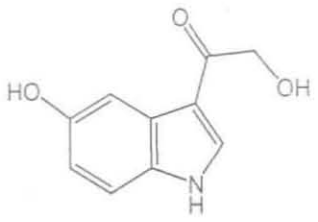


**5a**, R = α-OH
**5b**, R = ß-OH
**5c**, R = α-OAc

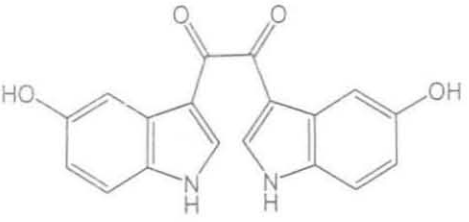


**6**

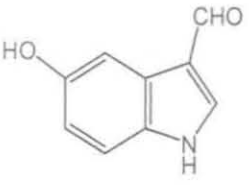
Steroids[5a] and indole alkaloids,[5] hyrtiosins A (**7**) and B (**8**) and 5-hydroxyindole-3-aldehyde (**9**) were isolated from ethyl acetate solubles of methanol extract of Okinawan *H. erecta*.[5d]



**7**

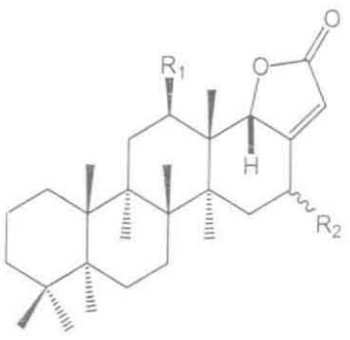


**8**



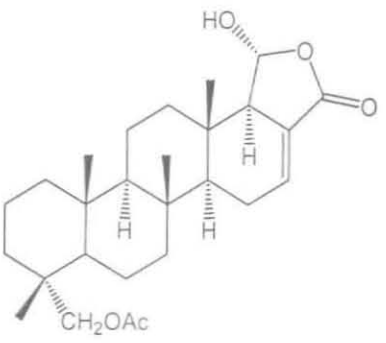
**9**

The carbon tetrachloride and dichloromethane solubles of ethanol extract of *H. cf. erectus*[6a] afforded cytotoxic pentacyclic sesterterpenes, 16-*O*-deacetyl-16-*epi*-scalarolbutenolide (**10**), 12-*O*-acetyl-16-*O*-deacetyl-16-*epi*-scalarolbutenolide (**11**)[6b] and 12-deacetoxy-21-acetoxyscalarin (**12**). A β-carbolin alkaloid, hyrtiomanzamine (**13**) was isolated from the methanol soluble portion of methanol-chloroform (1:1) extract of Red Sea New Caledonian variety having immunosuppressive acitivity.[7]



**10**, $R_1$ = OH, $R_2$ = ß-OH

**11**, $R_1$ = OAc, $R_2$ = ß-OH



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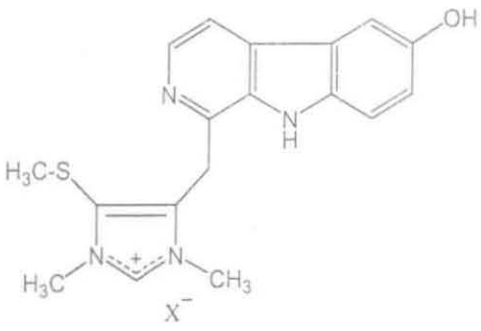
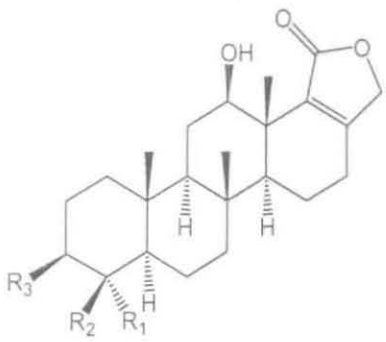


13

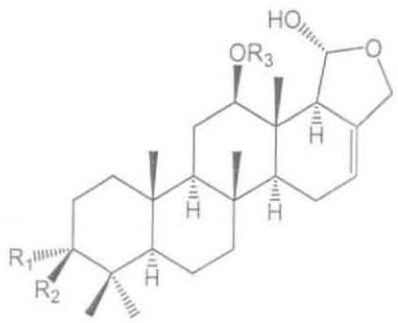


14, $R_1 = R_2 = CH_3$, $R_3 = OH$

15, $R_1 = CH_3$, $R_2 = CH_2OH$, $R_3 = H$

16, $R_1$ - $CH_2OH$, $R_2 = CH_3$, $R_3 = H$

Pettit *et al.* later found other types of potential biologically active compounds, like anticancer spongistatins,[8] sesterstatins 1-3 (**14-16**),[9] 15-oxopuupehenol (cancer cell line and malarial inhibitory),[10] dipuupehedione (cancer cell line inhibitory).[11a] The related metabolites were found to have antibacterial, antiviral, antifungal, cyto-toxic, and immunomodulatory activities by other workers.[11b,c]

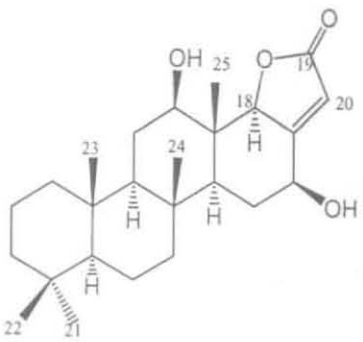


17, $R_1, R_2 = O$, $R_3 = H$

18a, $R_1 = R_3 = H$, $R_2 = OH$

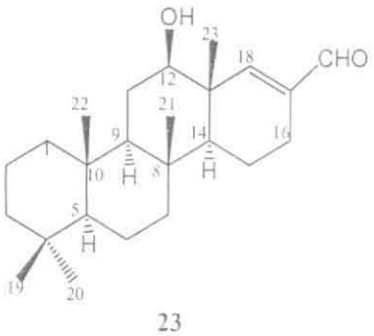
18b, $R_1 = H$, $R_2 = OH$, $R_3 = Ac$

19, $R_1 = R_2 = R_3 = H$

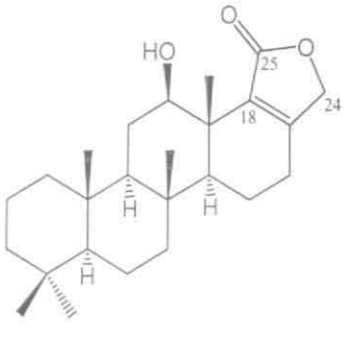


20

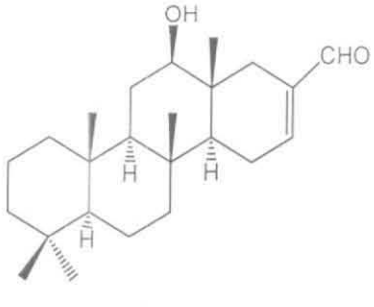
Sato *et al.* have isolated antitumour active scalarane sesterterpenes (**17-20**) from the ethyl acetate solubles of methanol extract of *H. erecta*[12] along with known altohyrtins. Yamada *et al.* work led to further isolation of this group of compounds[13] *viz.*, hyrtiolide (**21**), 16-hydroxyscalarolide (**22**), 12-deacetyl-$\Delta^{17}$-hyrtial (**23**), scalarolide[14] (**24**) and 12-deacetylhyrtial[15] (**25**) from the hexane and ethyl acetate soluble fraction of methanol extract of Okinawan *H. erecta*.




**23**

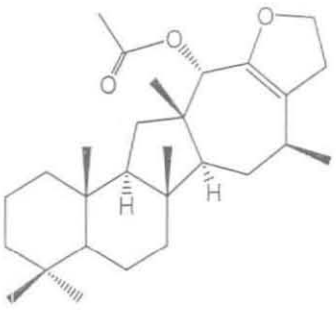



**24**

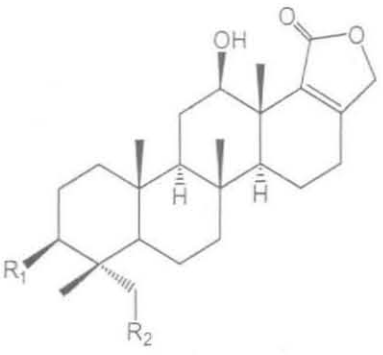



**25**

Scheuer *et al.*[16] have isolated further scalarane type sesterterpenes, salmahyrtisol-A (**26**), 3-acetylsesterstatin-1 (**27**), 19-acetyl-sesterstatin-3 (**28**), and salmahyrtisol-B (**29**) along with known compounds of hyrtiosal (**6**), scalarolide (**24**) and salmahyrtisol-C (**17**) from hexane solubles of methanol extract of Red Sea *H. erecta*. Further work on *Hyrtios* spp. led to isolation of other scalarane sesterterpenoids.[17]

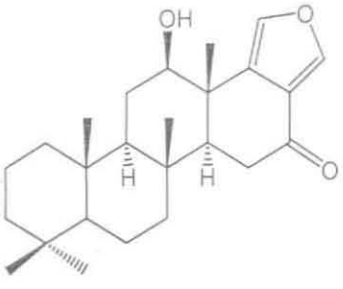


26

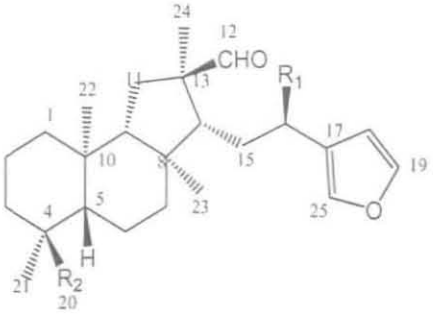


27, $R_1$ =H, $R_2$ = $OCOCH_3$

28, $R_1$ = $OCOCH_3$, $R_2$ = H



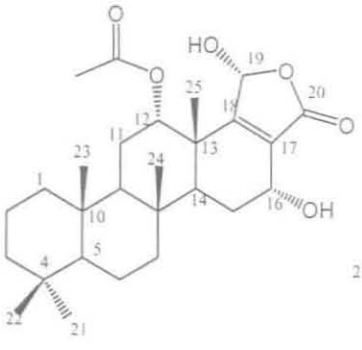
29



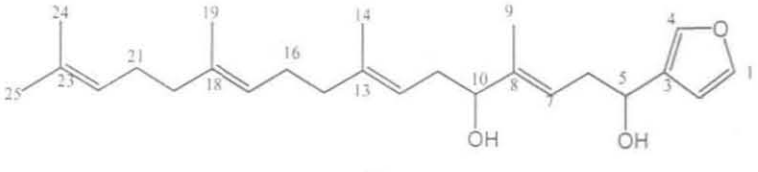
30, $R_1$ = OH, $R_2$ = CHO

31, $R_1$ = $OCOCH_3$, $R_2$ = CHO

Recently four new compounds[18] (**30-33**) have been isolated from methanol - chloroform extract of Chinese specimen of *H. erectus* along with seven known compounds.



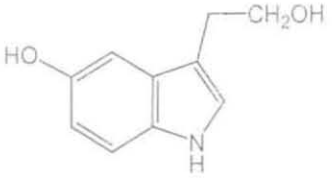
32



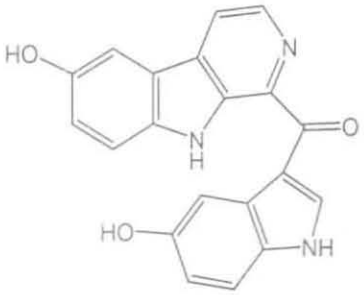
33

Braekman *et al.* isolated several sesquiterpene/quinines[19a] and four tryptamine derived alkaloids[19b] from dichloromethane soluble portion of methanol -

dichloromethane extract of *H. erectus*, of which two were the known compounds (**8**) and (**9**)[5d] along with two new 5-hydroxy-3(2-hydroxyethyl)-indole (**34**) and hyrtiosulawesine (**35**).

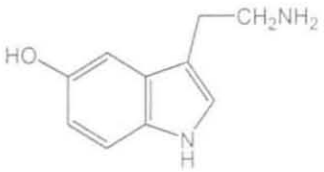



34

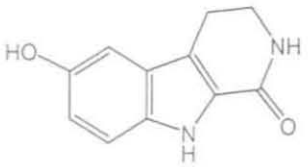



35

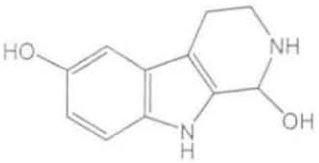
Two novel aplysinopsin-type indole alkaloids, and three known indole alkaloids were isolated from *H. erecta*.[20] Indole alkaloids, *β*-carboline,[19b] serotonin (**36**), 6-hydroxy-3,4-dihydro-1-oxo- *β*-carboline (**37**) and 1,6-dihydroxy-1,2,3,4-tetrahydro-*β*-carboline (**38**) were isolated from *H. reticulates*.




36



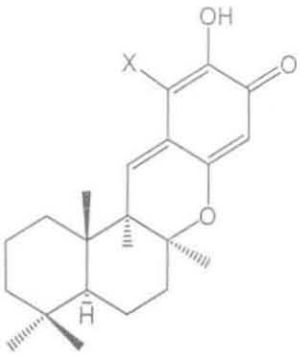

37




38

Many unidentified species of this genus have yielded few more new compounds of which puupehenone is of major occurrence. Puupehenone and its congeners are a distinctive class of merosesquiterpenes compounds like ilimaquinone. They are sesquiterpenes joined to a $C_6$-shikimate moiety. They have property to inhibit replication of the HIV virus[21] in addition to varied bioactivities. Scheuer *et al.* isolated puupehenone (**39**) related compounds (**40 - 45**), from Hawaiian sponge of *Hyrtios* spp.[22]

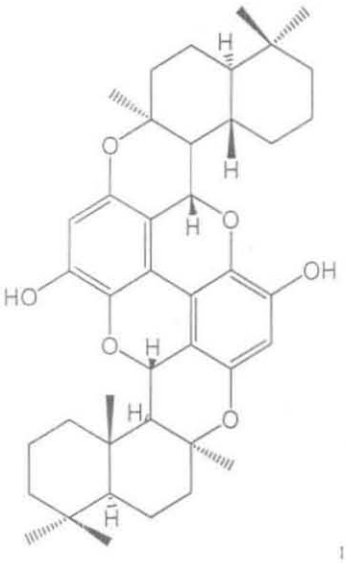
21-Halo derivatives of puupehenone (**40, 41**), bispuupehenone (**42**),[22a] 21-chloropuupehenol (**43**), 15-oxopuupehenol (**44**) and molokinenone (**45**) exhibited differential antitumor, antiviral and antimalarial activities.



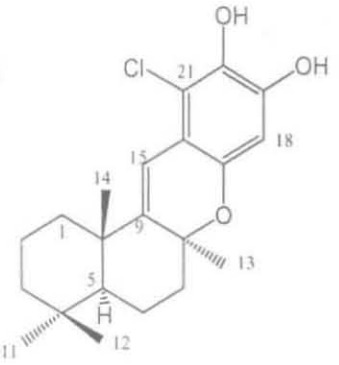
**39**, X = H

**40**, X = Cl

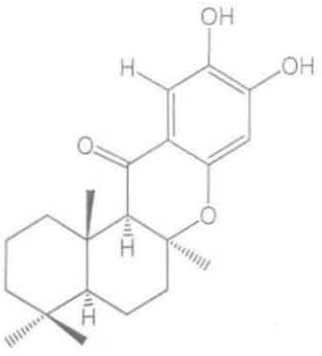
**41**, X =Br



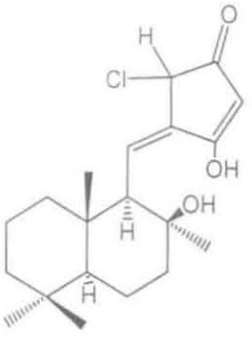
**42**



**43**

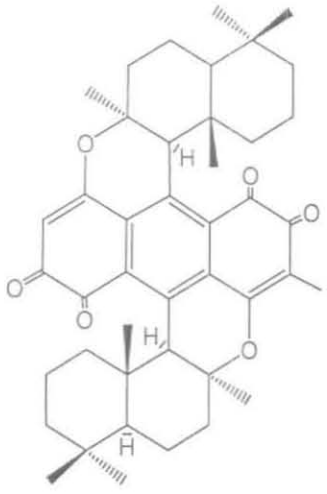


**44**

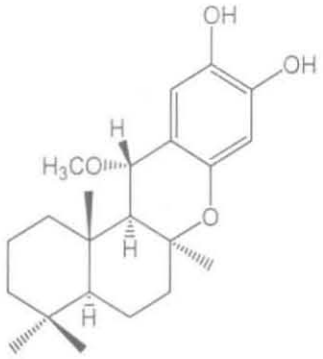


**45**

Kondracki *et al.* isolated a cytotoxic new red dimer of puupehenone, dipuupehedione (**46**) from dichloromethane soluble portion of one sponge[23a] and other biologically active sesterterpenes of the manoalide family, thorectolide monoacetate co-occurring with thorectolide from another specimen[23b] of New Caledonian *Hyrtios* spp.

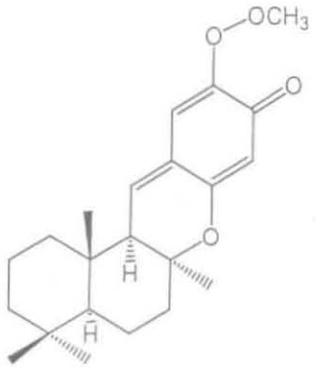


46

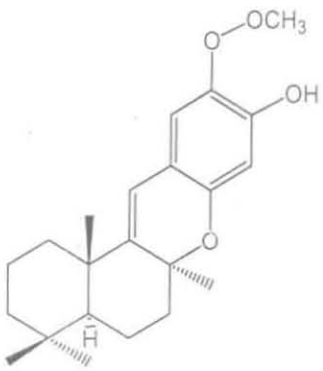


47

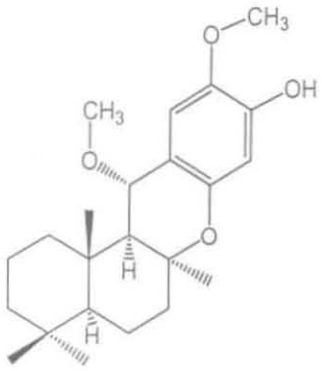
A methoxy derivative of 15α-methoxypuupehenol (**47**)[23c] having comparable biological activity with (**39**) was obtained during the course of extraction using methanol. Crews *et al.* isolated three methoxy derivatives of puupehenone (**48-50**)[24] from the dichloromethane solubles of ethyl alcohol extract of Indonesian specimen with absolute stereochemistry as *5*S,*8*S,*9*R,*10*S for the new compounds.



48

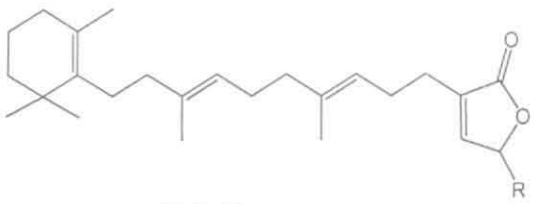


49

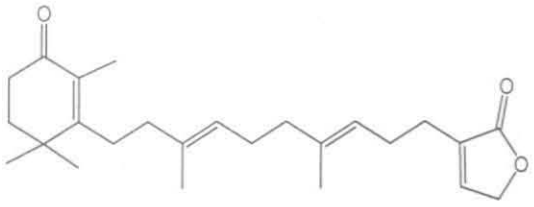


50

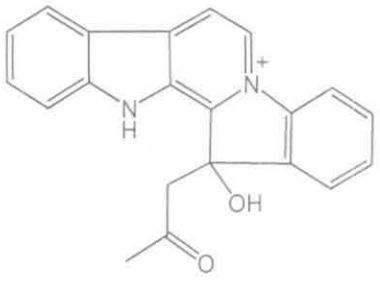
Two new sesterterpenes (**51** and **52**) and the known compounds isodehydroluffariellolide (**53**), homofascaplysin-A (**54**), and fascaplysin (**55**) were also encountered during the isolation from dichloromethane extract of *H. erecta.*[17b]



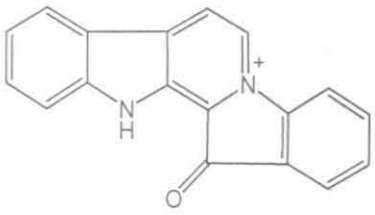
51, R = H
52, R = $OCH_2CH_3$



53

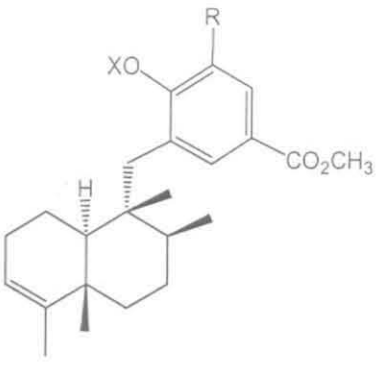


54

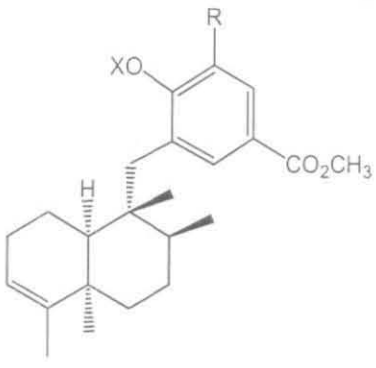


55

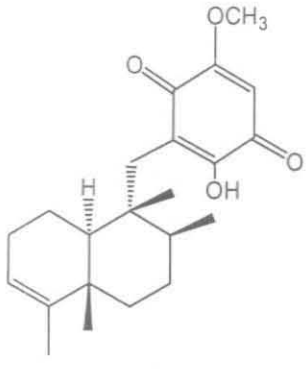
Sesquiterpene/quinines with 4,9-friedodrim-3-ene skeleton hyrtiophenol (**56**), 5-epihyrtiophenol (**57**), 18-hydroxy-5-epihyrtiophenol (**58**), and 18-hydroxy hyrtiophenol (**59**)] along with known isospongiaquinone (**60**) were isolated from *Hyrtios* spp. and 21-hydroxy-19-methoxyarenarone (**61**), which bears the 4,9-friedodrim-4(15)-ene skeleton, was isolated from *Hyrtios tubulatus* (Curacü ao) along with arenarol (**62**) and 5-epiilimaquinone (**63**) from dichloromethane soluble portion of methanol extract.[19a]



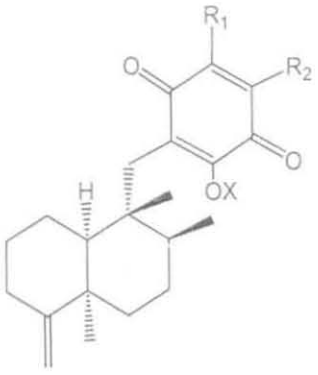
56, R = H, X = H
59, R = OH, X = H



57, R = H, X = H
58, R = OH, X = H

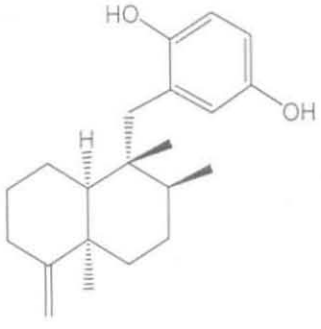


60




61, $R_1$ = H, $R_2$ = $OCH_3$, X = H

63, $R_1$ = $OCH_3$, $R_2$ = H, X = H




62

**Chemical Analysis of Sponge *Hyrtios* spp.**

| | | |
|---|---|---|
| Phylum | - | Porifera |
| Class | - | Demospongia |
| Sub class | - | Ceractinomorpha |
| Order | - | Dictyoceratida (Minchin1900) |
| Family | - | Thorectidae (Bergquist 1978) |
| Genus | - | *Hyrtios* spp. (Duchassaing & Michelotti 1864) |

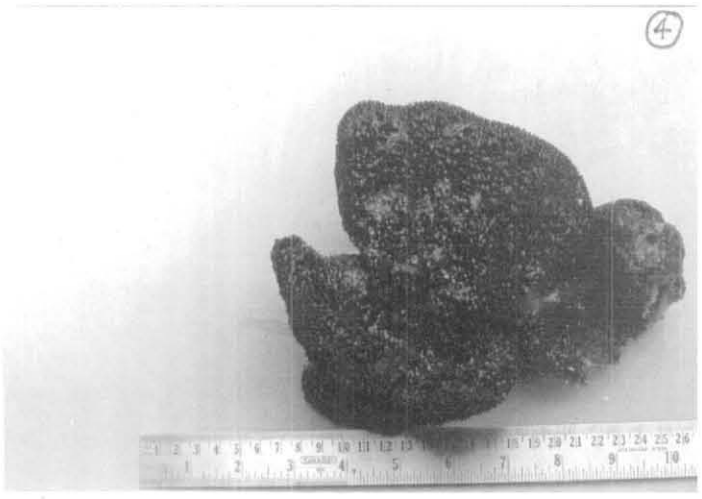
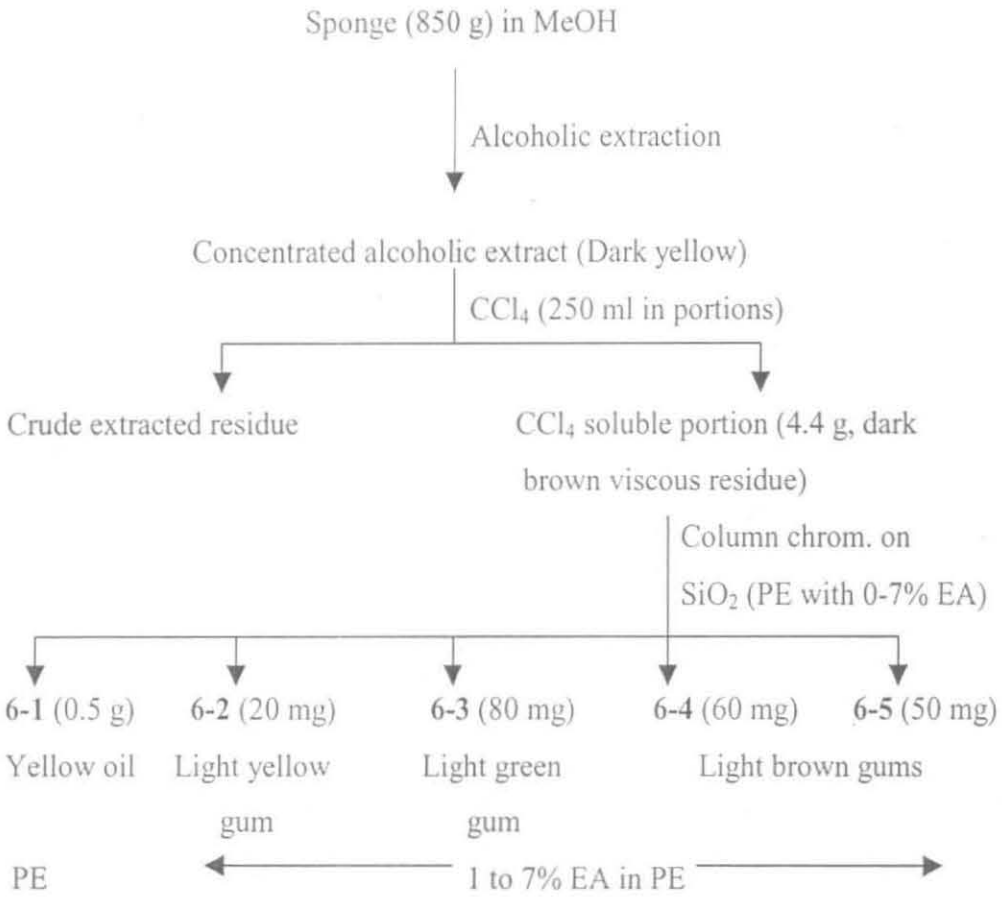


**Fig - 1**

The methanol (~600 ml) used to preserve the sponge *Hyrtios* spp. (**Fig - 1**) (850 g wet weight) was decanted and the sponge pieces were extracted thrice with ethyl alcohol (650 ml each). The alcoholic extracts were combined, filtered and concentrated. The concentrated extract was fractionated with carbon tetrachloride to recover a fraction. This fraction was column chromatographed over silica gel using petroleum ether with increasing concentration of ethyl acetate (0 to 7%) to collect four semi pure fractions (*tlc* monitoring) of **6-1, 6-2, 6-3** and **6-4**. This sponge offered semi pure residues as oils/gums with no solid fraction. Fraction **6-1** had high quantity of oily matter having the methyl esters of long chain fatty acids, as isolable by silica gel column. Each fraction on analysis by GC-MS revealed the nature of compounds present in them. The flow diagram of the extraction is depicted in **Scheme - 1**.




**Scheme-1**

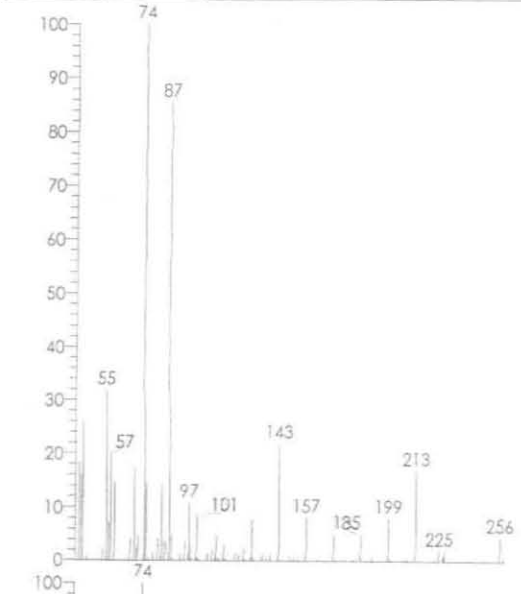
Analysis of fraction **6-1 (Table - 1)**

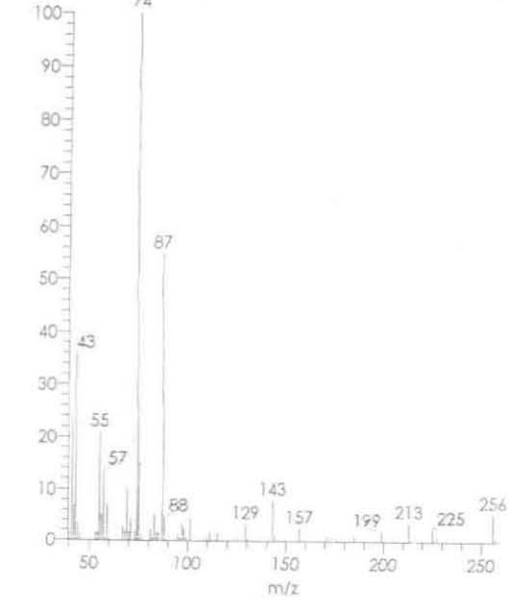
This fraction is obtained as the least polar fraction eluted by petroleum ether only and forms the major fraction of alcoholic extract because most of the crude extract of this sponge contains mainly oily matter. GC-MS analysis showed the presence of only methyl esters of both saturated and unsaturated LC fatty acids from chain length of $C_{14}$ to $C_{23}$. Fifteen compounds have been identified [**6-1-1, 6-1-2 (Fig – 2), 6-1-3, 6-1-4 (Fig - 3), 6-1-5 (Fig - 4), 6-1-6** to **6-1-9 (Fig - 5), 6-1-10** to **6-1-15**]. $C_{15}$, $C_{16}$, $C_{17}$ and $C_{19}$ acid esters contribute substantially in this mixture with composition of 10% and above. Other fatty acid esters contribution is below 10%.

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 821 | 858 | Pentadecanoic acid, m | |
| 2 | 819 | 825 | Pentadecanoic acid, m | |
| 3 | 795 | 859 | Pentadecanoic acid, m | |
| 4 | 757 | 757 | Tetradecanoic acid, 12-I | |
| 5 | 755 | 791 | Methyl tetradecanoate | |
| 6 | 749 | 816 | Tridecanoic acid, methy | |
| 7 | 748 | 771 | Hexadecanoic acid, me | |
| 8 | 748 | 864 | Tridecanoic acid, methy | |
| 9 | 746 | 812 | Tridecanoic acid, methy | |
| 10 | 742 | 773 | 9-Octadecenoic acid, 1 | |
| 11 | 740 | 746 | Pentadecanoic acid, m | |
| 12 | 738 | 768 | Methyl tetradecanoate | |
| 13 | 737 | 846 | Methyl tetradecanoate | |
| 14 | 736 | 761 | Methyl tetradecanoate | |
| 15 | 735 | 757 | Hexadecanoic acid, me | |
| 16 | 734 | 745 | Nonadecanoic acid, me | |
| 17 | 732 | 757 | Hexadecanoic acid, me | |
| 18 | 731 | 748 | Octadecanoic acid, me | |

Pentadecanoic acid, methyl ester
Formula C16H32O2, MW 256, CAS# 7132-64-1, Entry# 7578
Methyl n-pentadecanoate





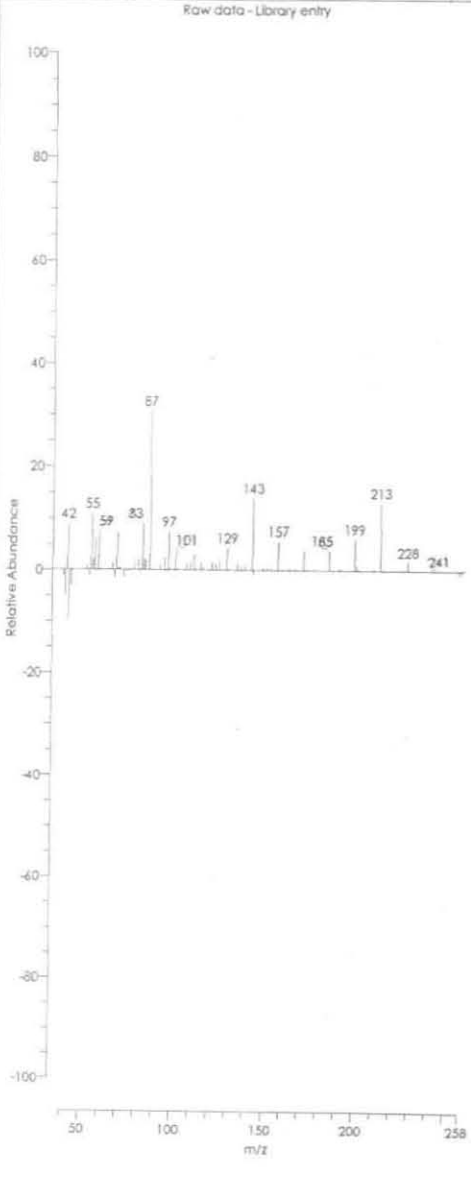
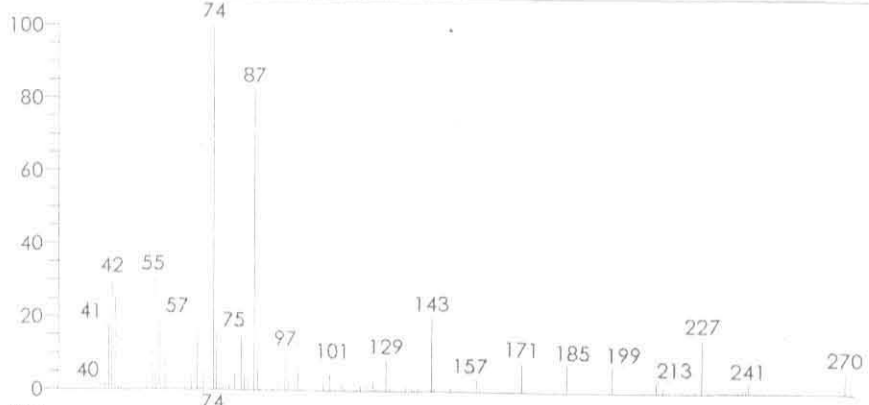



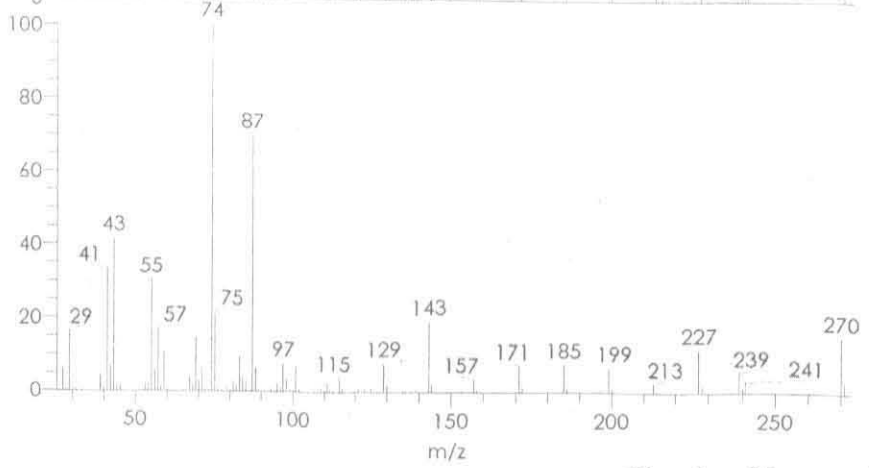

Fig – 2 Mass spectrum of 6-1-2

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 813 | 828 | Hexadecanoic acid, methyl este | |
| 2 | 812 | 818 | Hexadecanoic acid, methyl este | |
| 3 | 809 | 814 | Hexadecanoic acid, methyl este | |
| 4 | 800 | 819 | 9-Octadecenoic acid, 12-(acety | |
| 5 | 785 | 788 | Hexadecanoic acid, methyl este | |
| 6 | 770 | 791 | Hexadecanoic acid, methyl este | |
| 7 | 762 | 790 | Pentadecanoic acid, 14-methyl- | |
| 8 | 752 | 785 | Pentadecanoic acid, methyl este | |
| 9 | 749 | 803 | Pentadecanoic acid, methyl este | |
| 10 | 738 | 750 | Nonadecanoic acid, methyl este | |
| 11 | 738 | 738 | Pentadecanoic acid, 14-methyl- | |
| 12 | 738 | 812 | Tridecanoic acid, methyl ester | |
| 13 | 733 | 759 | Heptadecanoic acid, methyl est | |
| 14 | 732 | 804 | Tridecanoic acid, methyl ester | |

Hexadecanoic acid, methyl ester
Formula C17H34O2, MW 270, CAS# 112-39-0, Entry# 28354
Palmitic acid, methyl ester




4-1#1157 RT: 10.47 AV: 1 NL: 1.51E6 T: {0,0} + c EI det=350.00 Full ms [ 40.00-600.00]




SI 809, RSI 814, MAINLIB, Entry# 28354, CAS# 112-39-0, Hexadecanoic acid, methyl ester

Raw data - Library entry

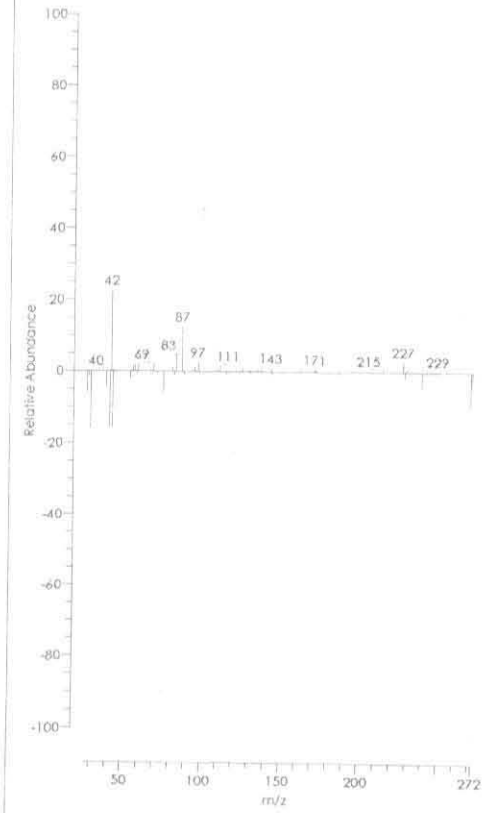
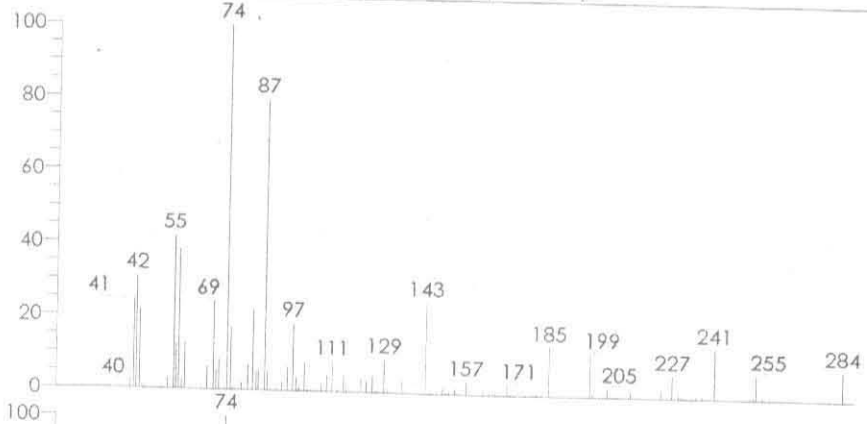


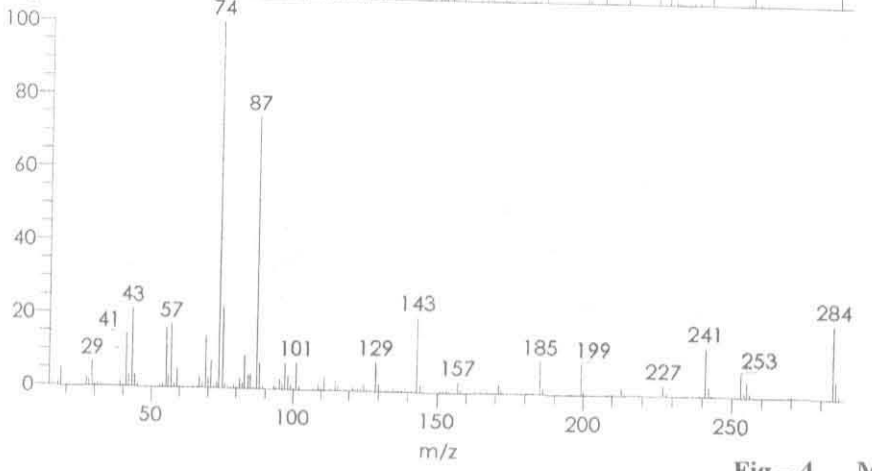

Fig – 3 Mass spectrum of 6-1-4

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 775 | 790 | Heptadecanoic acid, methyl ester | |
| 2 | 758 | 758 | Hexadecanoic acid, 14-methyl-, meth | |
| 3 | 750 | 761 | Heptadecanoic acid, methyl ester | |
| 4 | 739 | 747 | Heptadecanoic acid, methyl ester | |
| 5 | 729 | 733 | Hexadecanoic acid, 15-methyl-, meth | |
| 6 | 728 | 733 | Heptadecanoic acid, methyl ester | |
| 7 | 725 | 741 | Heneicosanoic acid, methyl ester | |
| 8 | 722 | 743 | Hexadecanoic acid, 14-methyl-, meth | |
| 9 | 718 | 784 | 9-Octadecenoic acid, 12-(acetyloxy)-, | |
| 10 | 716 | 730 | Eicosanoic acid, methyl ester | |
| 11 | 714 | 740 | Eicosanoic acid, methyl ester | |
| 12 | 712 | 771 | Hexadecanoic acid, methyl ester | |
| 13 | 710 | 739 | Octadecanoic acid, methyl ester | |
| 14 | 707 | 770 | Hexadecanoic acid, 15-methyl-, meth | |

Heptadecanoic acid, methyl ester
Formula C18H36O2, MW 284, CAS# 1731-92-6, Entry# 7596
Margaric acid methyl ester




4-1#1286 RT: 11.58 AV: 1 NL: 1.37E6 T: {0,0} + c EI det=350.00 Full ms [ 40.00-600.00]




SI 750, RSI 761, REPLIB, Entry# 7596, CAS# 1731-92-6, Heptadecanoic acid, methyl ester

Raw data - Library entry

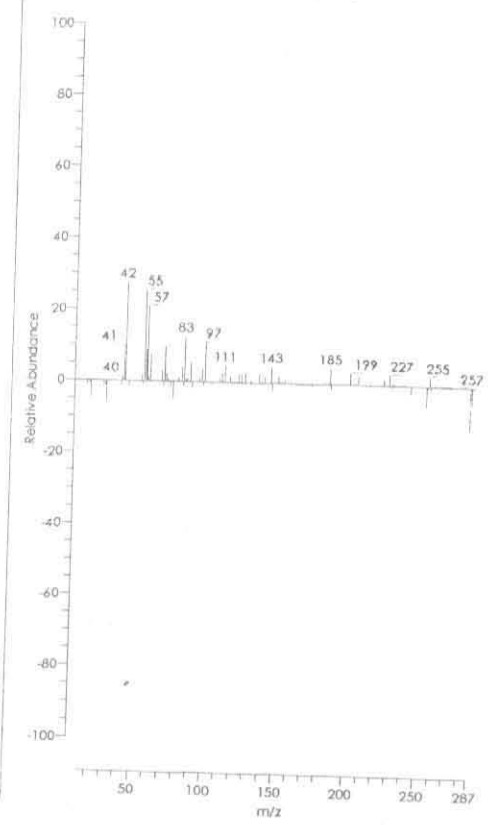
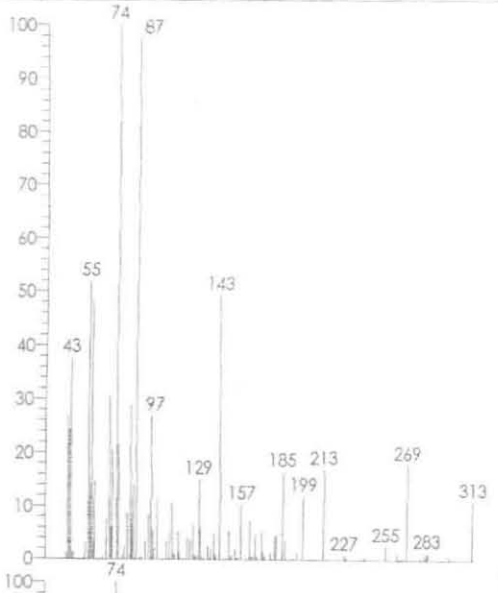
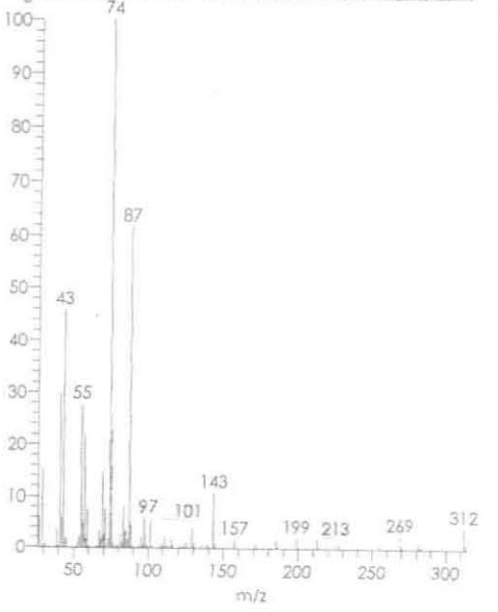



**Fig – 4 Mass spectrum of 6-1-5**

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 723 | 744 | Nonadecanoic acid, me | |
| 2 | 695 | 699 | Nonadecanoic acid, me | |
| 3 | 692 | 750 | Octadecanoic acid, me | |
| 4 | 692 | 750 | Octadecanoic acid, me | |
| 5 | 685 | 716 | Eicosanoic acid, methyl | |
| 6 | 678 | 685 | Octadecanoic acid, 17- | |
| 7 | 676 | 731 | Eicosanoic acid, methyl | |
| 8 | 676 | 710 | Octadecanoic acid, me | |
| 9 | 675 | 707 | Docosanoic acid, methy | |
| 10 | 674 | 690 | Nonadecanoic acid, me | |
| 11 | 672 | 676 | Octadecanoic acid, 10- | |
| 12 | 666 | 753 | Tetracosanoic acid, met | |
| 13 | 663 | 734 | Pentadecanoic acid, me | |
| 14 | 663 | 733 | Hexadecanoic acid, me | |
| 15 | 661 | 694 | Heneicosanoic acid, me | |
| 16 | 661 | 734 | Hexadecanoic acid, me | |
| 17 | 653 | 664 | Octadecanoic acid, 10- | |
| 18 | 653 | 752 | Tridecanoic acid, methy | |

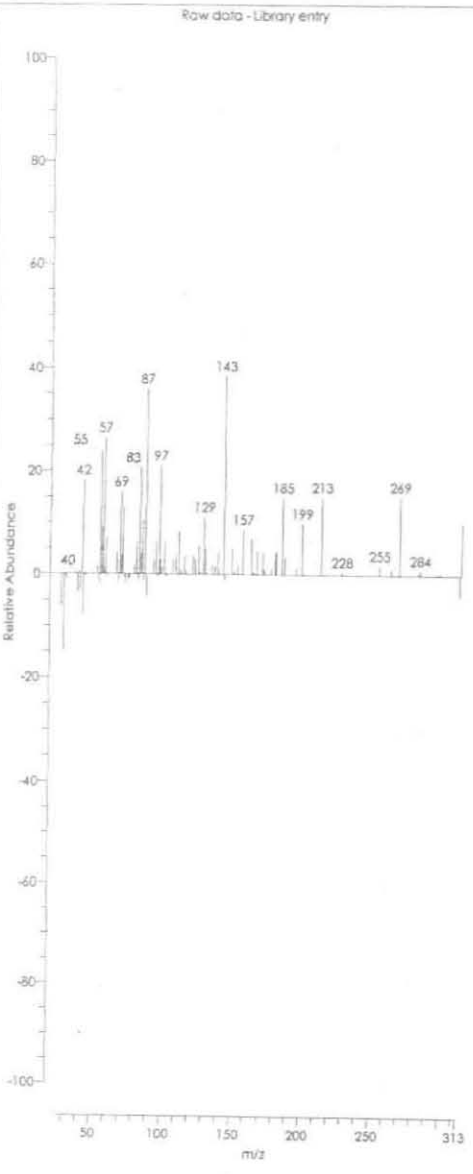




Fig – 5 Mass spectrum of 6-1-9

Most of the saturated esters show M+1 as the first MS peak. Base peak at *m/e* 74 is seen in most compounds arising out of McLafferty rearrangement accounting for fragment ion $[H_2C{=}C(OH)OMe]^+$ ($C_3H_6O_2$), as observed in fractions of other sponge extracts analyzed previously. Other prominent peaks and typical fragment pattern of each compound are indicative of the straight chain hydrocarbons. Compounds (**6-1-7**) and (**6-1-13**) show the base peak due to butene fragment ion, $[C_4H_7]^{+\cdot}$, along with other fragments with successive clusters differing by mass unit of *m/e* 14 in the mass spectrum as generally observed in straight chain hydrocarbons. The cleavage of $C_3$-$C_4$ bond to give *m/e* 87 for the fragment ion $[CH_2CH_2COOCH_3]^{+\cdot}$ is dominant in most of these compounds. Other prominent peaks characteristic of higher molecular weight and the details of molecular parameters of the compounds identified are given in **Table - 1.**

Analysis of fraction **6-2 (Table - 2)**

The major component (**6-2-1**) with RT of 16.49 has been identified as dioctyl phthalate. Elimination of one alkyl $C_8H_{17}$ unit gives fragment at *m/e* 280 as the first *ms* peak and subsequent cleavage of 2$^{nd}$ alkyl unit gives *m/e* 167 followed by the ready elimination of water molecule to give rise to intense peak at *m/e* 149 accounting for phthalic anhydride. Other minor compounds in this fraction could not be characterized (**Table - 2**).

Analysis of fraction **6-3 (Table - 3)**

Two compounds [**6-3-1** and **6-3-2** (**Fig – 6**)] have been identified in this fraction. Compound **6-3-1** has M+ ion and another weak peak at *m/e* 236 formed by methyl group elimination. Subsequent terminal butene unit cleavage gives weak peak at *m/e* 181. Elimination of $CH_2OH$ gives peak at *m/e* 149 further. Other prominent peaks at *m/e* 69, 81, 109 and 124 can be attributed to $[(CH_3)_2C{:}CHCH_2]^{+\cdot}$, $[(CH_3)_2C{:}CHCHCH]^{+\cdot}$ ($C_6H_9$, devoid of 2Hs), $C_8H_{13}$ and $C_9H_{16}$ respectively (**Table - 3**).

Table-1: Analysis of fraction 6-1

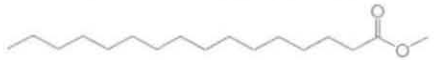
| Comp No. | RT | Peak Area %age | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|---|
| 6-1-1 | 8.29 | 3.02 | $C_{15}H_{30}O_2$ | 242 |  Methyl tetradecanoate | 243 | 42, 74, 87, 55, 199 |
| 6-1-2 | 9.45 | 12.68 | $C_{16}H_{32}O_2$ | 256 |  Methyl pentadecanoate | 258 | 74, 87, 42, 55, 143, 213 |
| 6-1-3 | 9.53 | 4.40 | $C_{16}H_{32}O_2$ | 256 |  Methyl 12-methyl tetradecanoate | 256 | 74, 87, 55, 199, 143, 41, 97, 213, 227 |
| 6-1-4 | 10.47 | 20.87 | $C_{17}H_{34}O_2$ | 270 |  Methyl hexadecanoate | 270 | 74, 87, 42, 55, 143, 227 |
| 6-1-5 | 11.58 | 14.34 | $C_{18}H_{36}O_2$ | 284 |  Methyl heptadecanoate | 285 | 74, 87, 55, 143, 43, 97, 241 |
| 6-1-6 | 11.69 | 2.26 | $C_{18}H_{36}O_2$ | 284 |  Methyl 14-methyl hexadecanoate | 285 | 74, 87, 42, 143, 55, 69, 97, 241 |

**Table-1 contd...**

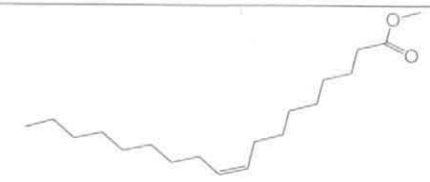
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **6-1-7** | 12.65 | 7.19 | $C_{19}H_{36}O_2$ | 296 |  Methyl 9(Z)-octadecenoate | 296 | 55, 74, 87, 96, 41, 264, 180, 137 |
| **6-1-8** | 12.81 | 5.05 | $C_{19}H_{38}O_2$ | 298 |  Methyl octadecanoate | 299 | 74, 87, 55, 143, 42, 255, 199 |
| **6-1-9** | 13.21 | 11.57 | $C_{20}H_{40}O_2$ | 312 |  Methyl nonadecanoate | 313 | 74, 87, 55, 43, 97, 143, 269, 213, 185 |
| **6-1-10** | 13.62 | 5.36 | $C_{20}H_{38}O_2$ | 310 |  Methyl 2-octyl cyclopropaneoctanoate | | 55, 74, 87, 42, 109, 123, 279 |
| **6-1-11** | 14.36 | 1.20 | $C_{21}H_{40}O_2$ | 324 |  Methyl 11-eicosenoate | 326 | 42, 74, 55, 87, 97,109, 143, 292 |
| **6-1-12** | 14.92 | 2.89 | $C_{22}H_{44}O_2$ | 340 |  Methyl heneicosanoate | 341 | 74, 42, 87, 55, 97, 143, 199 |

**Table-1 contd...**

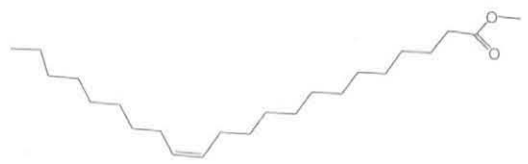
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **6-1-13** | 16.12 | 1.38 | $C_{23}H_{44}O_2$ | 352 |  Methyl 13(Z)-docosenoate | 352 | 55, 42, 69, 83, 97, 111, 321 |
| **6-1-14** | 16.24 | 1.29 | $C_{23}H_{46}O_2$ | 354 |  Methyl docosanoate | 355 | 74, 42, 87, 55, 143, 311, 255, 97 |
| **6-1-15** | 16.60 | 6.50 | $C_{24}H_{48}O_2$ | 368 |  Methyl tricosanoate | 369 | 74, 87, 57, 43, 143, 255, 326, 97, 199 |

**Table-2:** Analysis of fraction **6-2**

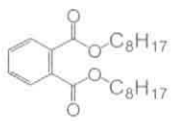
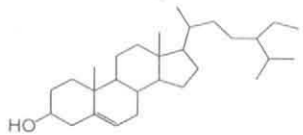
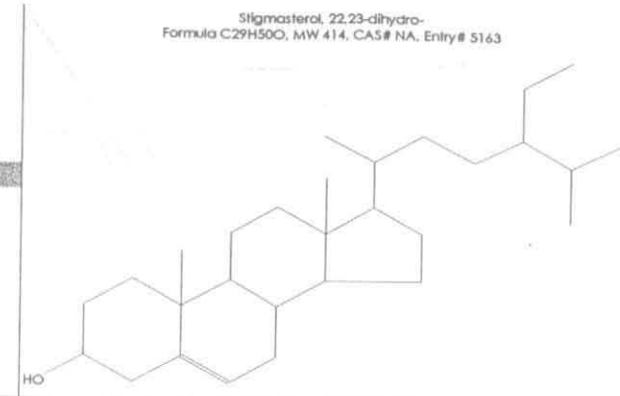
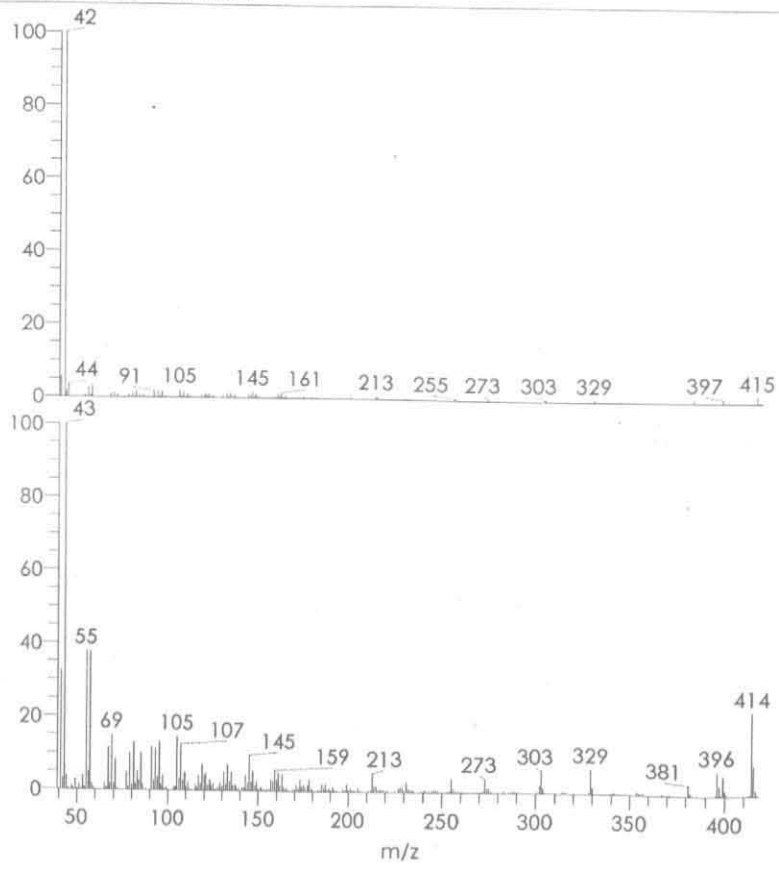
| Comp No. | RT | Peak area %age | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|---|
| **6-2-1** | 16.49 | 75.78 | $C_{24}H_{38}O_4$ | 390 |  Dioctylphthalate | 282 | 149, 42, 167 |

**Table-3:** Analysis of fraction **6-3**

| Comp No. | RT | Peak area %age | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|---|
| **6-3-1** | 11.74 | 25.79 | $C_{17}H_{30}O$ | 250 |  5,9,13-Trimethyl 4,8,12-tetradecatrienol-1 | 250 | 42, 109, 81, 124, 55 |
| **6-3-2** | 30.42 | 30.01 | $C_{29}H_{50}O$ | 414 |  T-Sitosterol | 415 | 42, 55, 105, 145 |

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 576 | 721 | Dihydrot | |
| 2 | 544 | 556 | τ-Sitoster | |
| 3 | 515 | 544 | Campes | |
| 4 | 510 | 602 | 20-Meth | |
| 5 | 484 | 484 | τ-Sitoster | |
| 6 | 478 | 478 | Stigmast | |
| 7 | 478 | 500 | Campes | |
| 8 | 466 | 466 | β-Sitoste | |
| 9 | 450 | 504 | Cholestc | |
| 10 | 447 | 459 | β-Sitoste | |
| 11 | 444 | 809 | Methane | |
| 12 | 444 | 444 | β-Sitoste | |
| 13 | 443 | 508 | Pregn-5- | |
| 14 | 442 | 513 | 13-Iso-ar | |




4-2#3472 RT: 30.42 AV: 1 NL: 3.27E5 T: {0,0} + c EI det=350.00 Full ms [ 40.00-600.00]

SI 478, RSI 478, MAINLIB, Entry# 5163, CAS# NA, Stigmasterol, 22,23-dihydro-

Raw data - Library entry

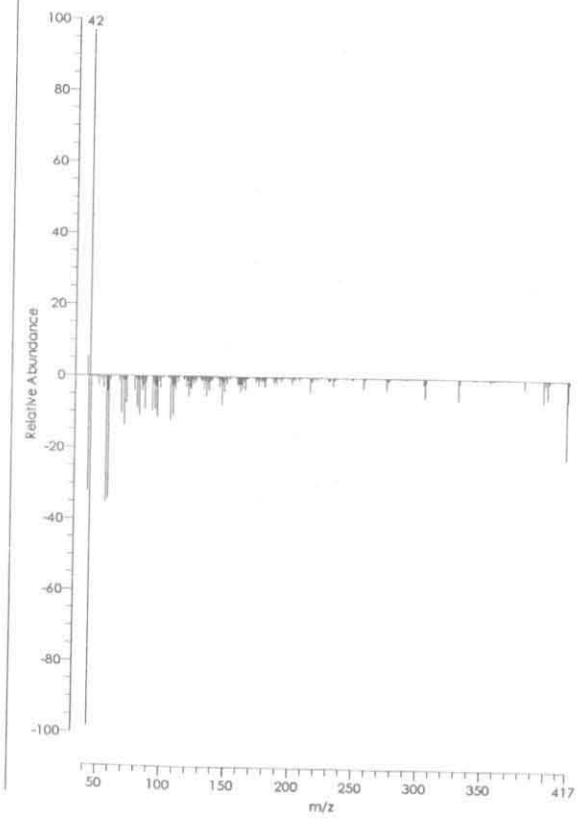
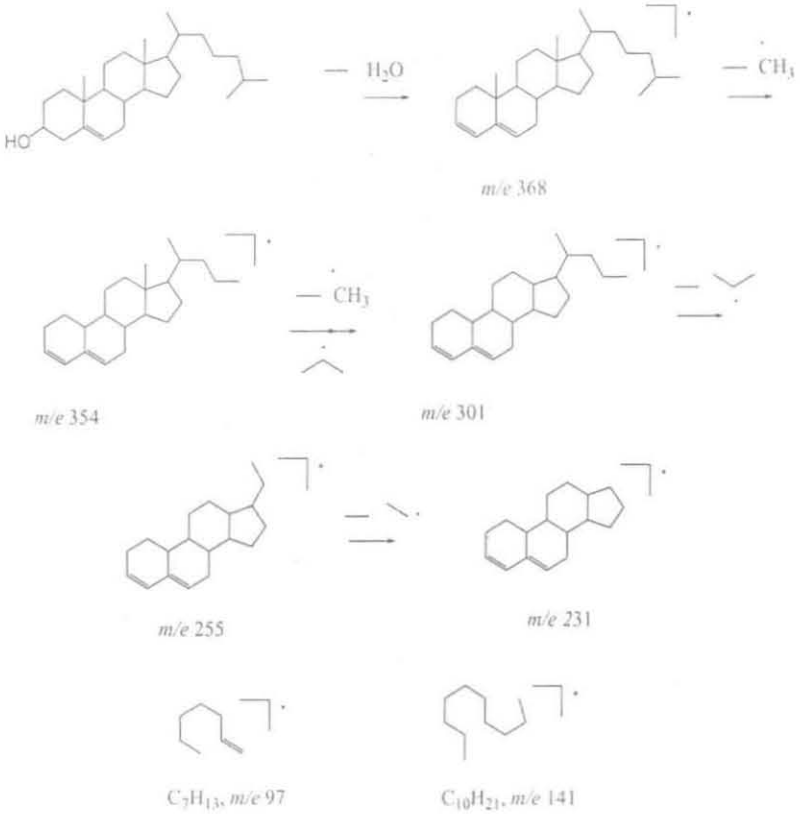



**Fig – 6 Mass spectrum of 6-3-2**

Analysis of fraction **6-4 (Table - 4)**

This fraction contains three steroids **6-4-1**, **6-4-2** & **6-4-3** *viz.* cholesterol, cholestanol and *β*-sitosterol respectively. Compound **6-4-3** contributes maximum composition while other two are almost equal about 8% each. The base peak appeared as 42 arising out of terminal propyl fragment, $[(CH_3)_2C]^{+\cdot}$. The first mass spectral peak appeared at M+3 for **6-4-1**, M+2 for **6-4-2** and M+1 & M+2 for **6-4-3** (arising as two peaks in GC). Elimination of water molecule gives rise to peak at 368. Other probable fragmentation pattern for other remarkable peaks may be rationalized as:




Compound **6-4-2**: It exhibits the similar fragmentation pattern as **6-4-3** with *m/e* at 370, 355, 301, 233, 257, etc with the absence of double bond at 5,6 position. Remaining small fragments attribute to the further fragmentation of the phenanthrane skeleton. For compound **6-4-3**, appearance of *m/e* at 396 by elimination of water, 381 by water and methyl groups, 42 by isopropyl group and 57 by butyl fragment ion (**Table - 4**).

Analysis of fraction 6-5 (**Table - 5**)

This last fraction has only steroids with 3 peaks in GC identified as cholesterol (**6-5-1**), cholest-7-en-3-ol (**6-5-2**) and $\tau$-sitosterol (**6-5-3**). The compositions of (**6-5-3**) is more than 50% and that of (**6-5-1**) & (**6-5-2**) are approximately equal (**Table - 5**).

**Table-4:** Analysis of fraction **6-4**

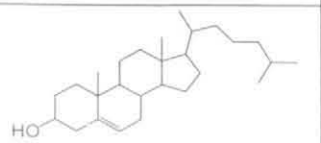
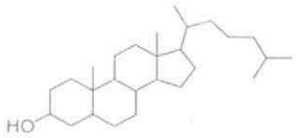
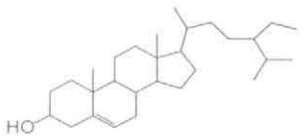
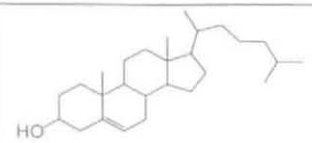
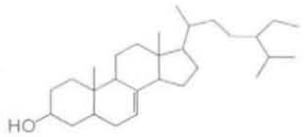
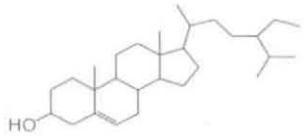
| Comp No. | RT | Peak area %age | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|---|
| **6-4-1** | 23.97 | 8.08 | $C_{27}H_{46}O$ | 386 |  Cholesterol | 389 | 42, 95, 107, 145, 159, 213, 255, 301, 368 |
| **6-4-2** | 24.09 | 7.89 | $C_{27}H_{48}O$ | 388 |  Cholestanol | 390 | 42, 215, 93, 370, 374, 355, 300, 233 |
| **6-4-3** | 29.58 | 84.03 | $C_{29}H_{50}O$ | 414 |  B-Sitosterol | 415 | 42, 396, 381, 329, 213, 145, 105, 57 |

**Table-5:** Analysis of fraction **6-5**

| Comp No. | RT | Peak area %age | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|---|
| **6-5-1** | 23.91 | 22.10 | $C_{27}H_{46}O$ | 386 |  Cholesterol | 388 | 42, 145, 91, 213, 255, 301, 368 |
| **6-5-2** | 25.21 | 24.31 | $C_{27}H_{46}O$ | 386 |  Cholest-7-en-3-ol | 388 | 42, 255, 105, 91, 55 |
| **6-5-3** | 30.39 | 53.59 | $C_{29}H_{50}O$ | 414 |  T-Sitosterol | 416 | 42, 57, 145, 213, 255, 329, 397 |

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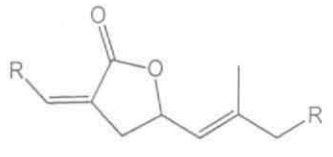
Chapter 7

# Chemical investigation of the sponge *Spongia* spp.

**Compounds of sponge genus *Spongia* spp.**

The genus *Spongia* spp. has been extensively studied to record a good array of structurally diverse metabolites. Most of the compounds are however, mevalonate derived, suggesting a biosynthetic origin rather than a symbiotic source. Furanoterpenes, nitenin (**1**) and dihydronitenin (**2**)[1a,b] were isolated from acetone soluble portion of *S. agaricina* of Italy and Spain samples[1c] along with 9,11-secoster-ols (**6**, **7**, **10**), pentacyclic sesterterpenoids called scalarins (**8**, **9**, **11**), 12-deacetyl-12,18-di-*epi*-scalaradial (**12**), 12-*epi*-scalaradial(12-$\beta$) (**13**), 12-*epi*-scalarin (**14**), and 12-*epi*-deoxoscalarin (**15**), furospinulosin-1 (**4**), anhydrofurospongin-1 (**5**) and isonitenin (**3**).

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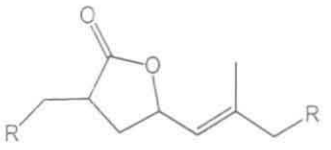


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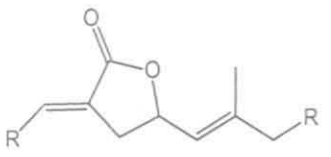
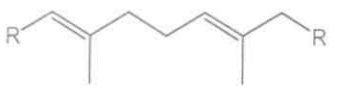



3


4

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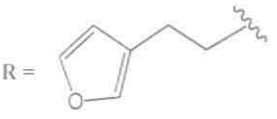


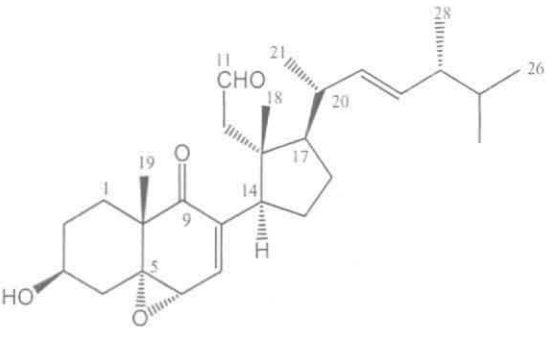


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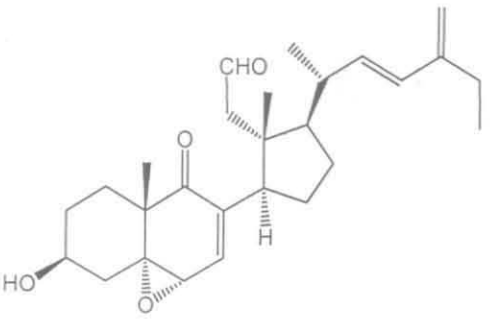


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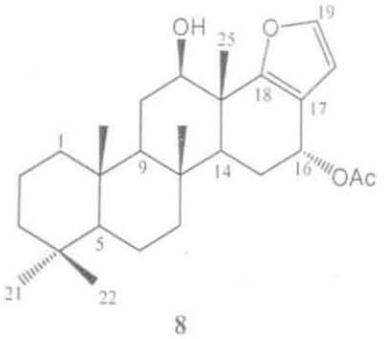


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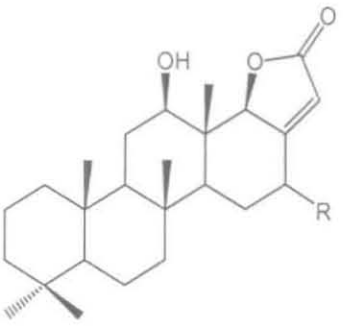
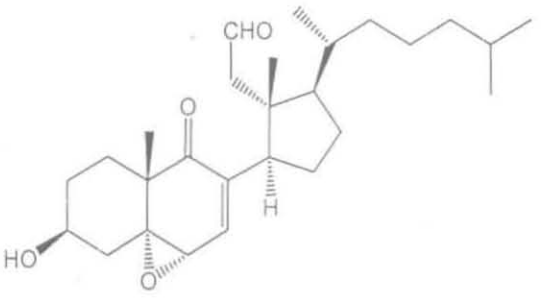
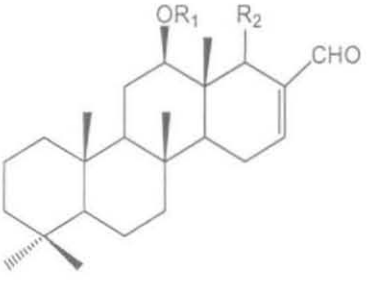



9, R = ß-OAc
11, R = α-OAc

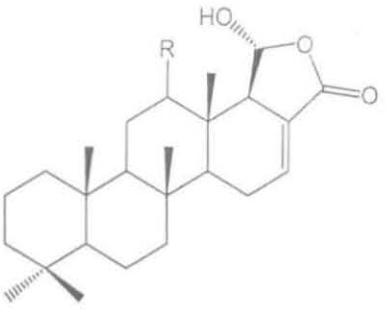



10



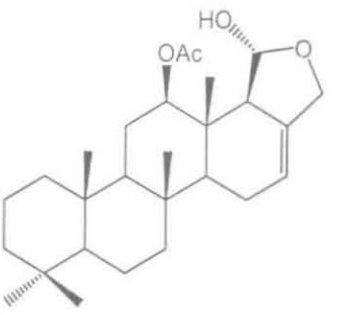

12, $R_1$ = H, $R_2$ = α-CHO

13, $R_1$ = H, $R_2$ = ß- CHO




14, R = ß-OAc

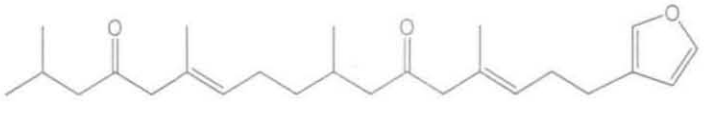
22, R = α–OAc




15

Faulkner *et al.*[2b] isolated linear sesterterpenes, furospinosulin-1 (**4**) and idiadione (**16**) and the scalarins, heteronemin (**17**), 12-*epi*-deoxoscalarin (**15**),

12-deacetyl-12,18-di-*epi*-scalaradial (**12**), scalarafuran (**18**) and scalarolide (**19**) from hexane soluble material of *S. nitens*.

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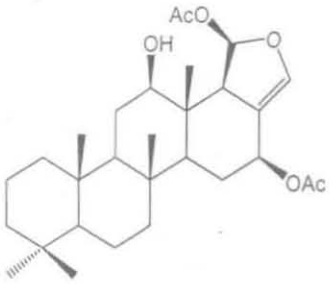


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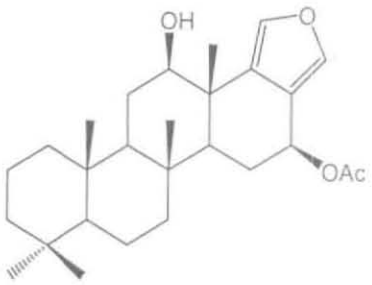


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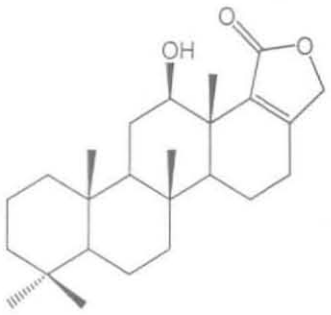
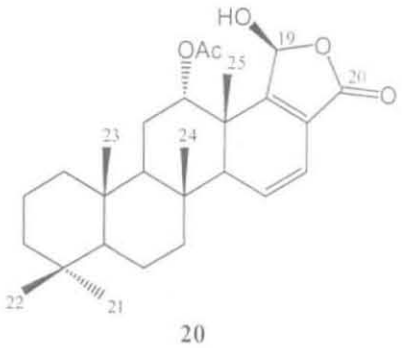
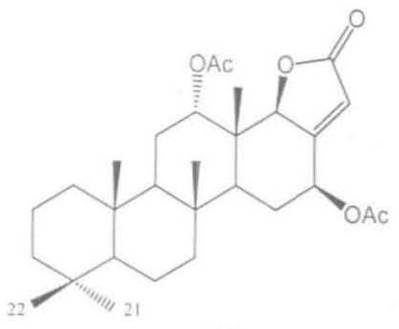



19

From dichloromethane soluble portion of methanol extract of Palauan sponge of *S. matamata*[3] novel scalarane class of compounds, 12$\alpha$-acetoxy-19$\beta$-hydroxyscalara-15,17-dien-20,19-olide (**20**), 12$\alpha$,16$\beta$-diacetoxyscalarolbutenolide (**21**), 9,11-secosterol, and 3$\beta$-hydroxy-5$\alpha$,6$\alpha$-epoxy-9-oxo-9,11-seco-5$\alpha$-cholest-7-en-11-al (**10**) along with scalarin (**22**) have been isolated.

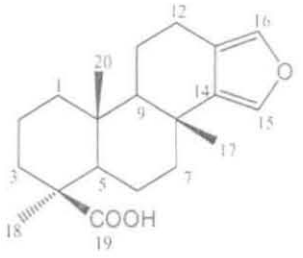



20

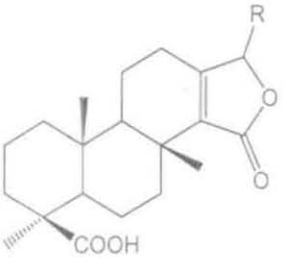



21

Schmitz *et al.*[4] isolated spongians, tetracyclic diterpenoids (**23-29**), from dichloromethane soluble portion of methanol extract of Micronesian specimen of *S. matamata* some of which exhibited antiviral and cytotoxic activities.



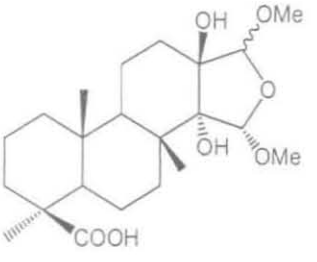

**23**



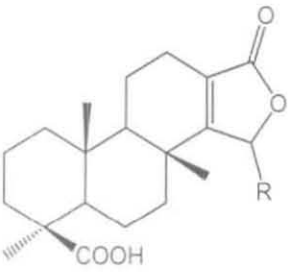

**24**, R = ß-OMe

**25**, R = α–OMe

**28**, R = H



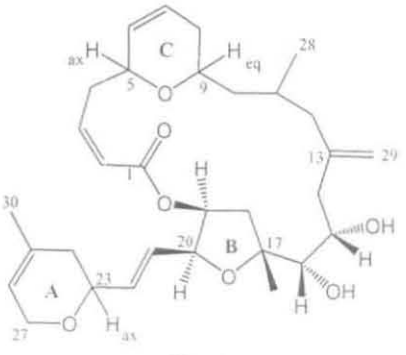

**27**



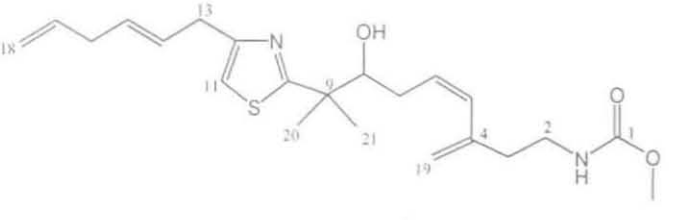

**26**, R = α–OMe

**29**, R = H

Crews *et al.* isolated two cytotoxic macrocyclic lactones, fijianolides A (**30**) and B[5a] and an unusual thiazole containing compound, mycothiazole[5b] (**31**) from dichloromethane soluble fraction of methanol extract of from Vanuatu specimens of *S. mycofijiensis*.

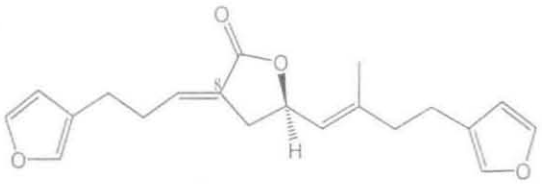



**30**

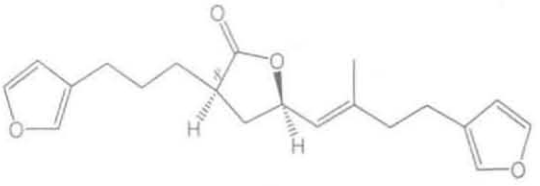



**31**

Compounds **30** and **31** were found to exhibit cytotoxic and anthelminthic (*in vitro*) activity respectively. Fattorusso *et al.* isolated $C_{21}$ furanoterpenes, nitenin (**32**) and dihydronitenin (**33**)[6] from ether soluble fraction of methanol extract of Mediterranean specimen, *S. nitens*[6a] and acetone soluble of *S. agaricina*.[6d] The absolute stereochemistry has recently been further confirmed.[6d]

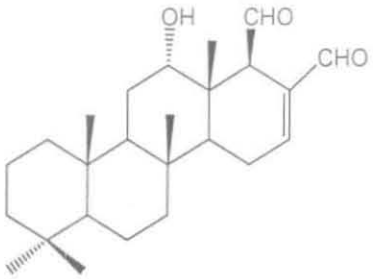


32

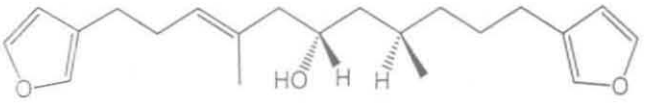


33

The same species afforded 12$\beta$- epimer of scalarin (**22**) and compound (**15**)[7] from ether soluble portion of its acetone extract. Deacetylscalarin (**34**), a $C_{12}$ epimer of (**13**) was found in dichloromethane soluble fraction of *S. oceania*.[8]

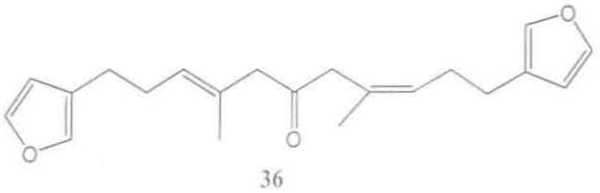



34

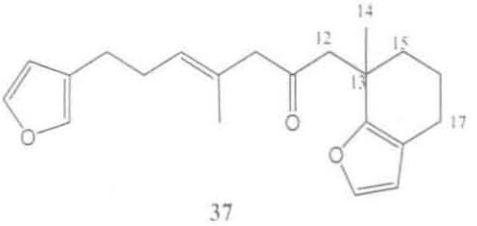



35

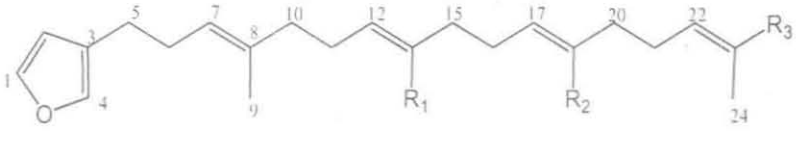
Furospongin-1 (**35**) related to (**32**) and (**33**) was isolated from ether soluble portion of methanol extract of *S. officinalis*.[9] Further derivatives and related $C_{21}$ furanoterpenes present in smaller amounts were isolated from the same sponge.[10] Recently Salva *et al.* isolated new furanoterpenes, furospongin-5 (**36**), cyclofurospongin-2 (**37**) and a furanosesterterpene, demethylfurospongin-4 (**38**) along with other terpenes previously reported *ie.*, isomer 1 of furospongin-2 (**39**), cyclofurospongin-2 (**40**), isomer 2 of furospongin-2 (**41**), isofurospongin-2 (**42**), dihydrofurospongin-2 (**43**), isomer 3 of furospongin-2 (**44**), furospongin-2 (**45**) and furospongin-4 (**46**). These compounds were obtained from the acetone extract of Spanish specimen.



36

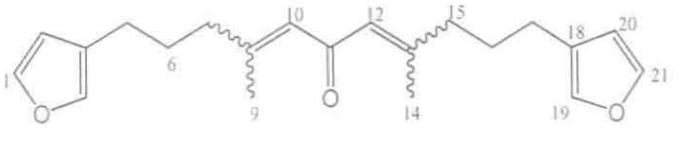


37



38, $R_1$ = Me, $R_2$ = COOH, $R_3$ = COOH

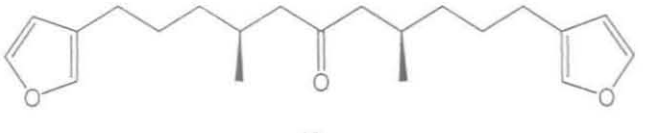
46, $R_1$ = Me, $R_2$ = COOH, $R_3$ = COOMe



39, 8Z, 12Z

41, 8Z, 12E

44, 8E, 12E



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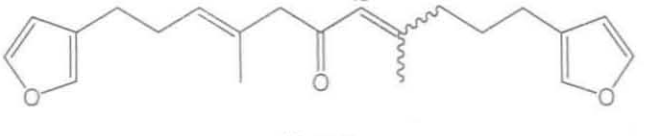
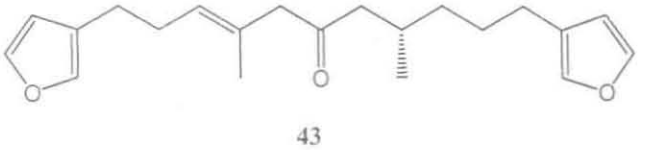


42, 12Z

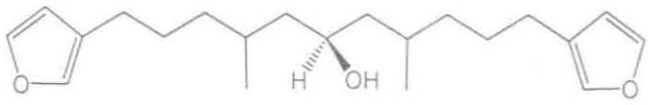
45, 12E



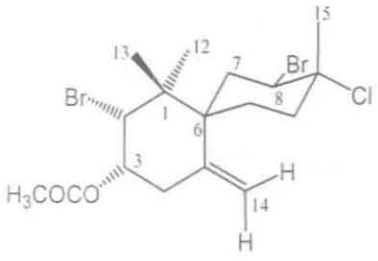
43

*S. officinalis* had been extensively studied, affording further furanosesterterpenes and C-21 furanoterpenes among its constituents.[11]

(+)-Untenospongin B (**47**), an enantiomer of its (-)-isomer was isolated from diethyl ether soluble fraction of acetone extract Mediterranean specimen of *S. virgultosa*.[6d]



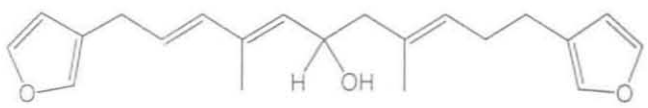

**47**



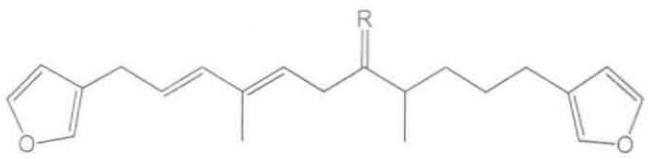

**48**

Halogenated *β*-Chamigrane type sesterterpene, rogiolol acetate [(+)-(2R,3S,6R, 8R,9R)-2,8-dibromo-9-chloro-1,1,9-trimethyl-5-ethylidenespiro[5,5]undec-3-yl acetate] (**48**)[12] the first of its kind generally found in red alga, *Laurencia* spp. was isolated from hexane soluble portion of ethanol extract of *S. zimocca*.

Kazlauskas *et al.* isolated tetrahydrofurospongin-1 (**49**),[6h] furospongenol (**50**) and furospongenone (**51**)[13] from cold petroleum ether extract of *Spongia* spp.



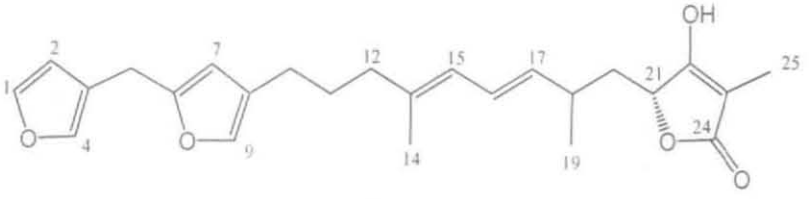

**49**



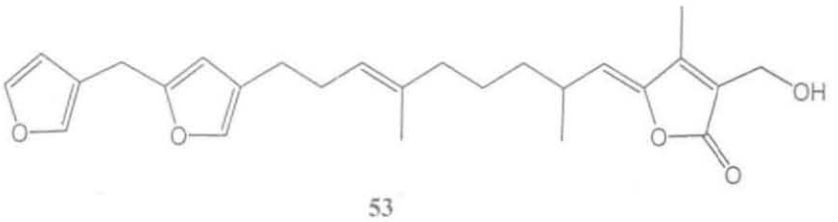

**50**, R = H, OH

**51**, R = O

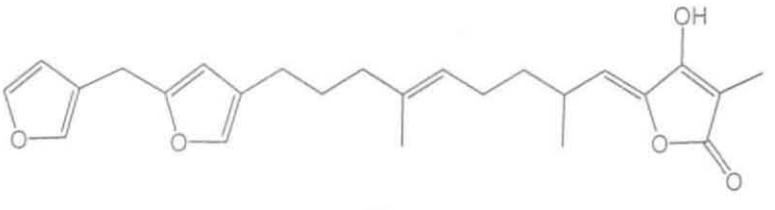
Anatiabacterial acyclic sesterterpenes with furano and tetronic acid termini, cometin-A (**52**), -B (**53**) and –C (**54**) were isolated by Capon *et al.*[14a] from ethanol extract of Australian *Spongia* spp. along with (**4**).




52

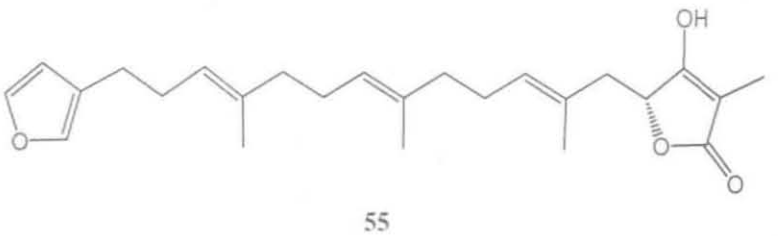



53

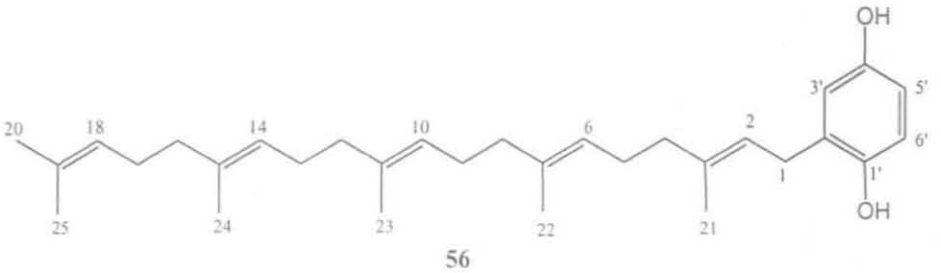



54

Further sesterterpene tetronic acid (**55**) and pentaprenylated *p*-quinol (**56**)[14b] were also isolated from ethanol extract the same genus.



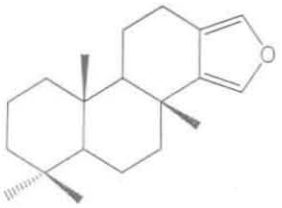

55



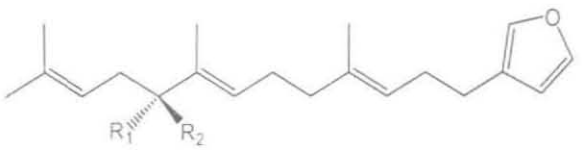

56

Spongia-13(16),14-diene (**57**)[15a], a member of tetracyclic spongian diterpenes was first isolated from *Spongia* spp. Later Molinski *et al.*[15b] isolated from the ethyl acetate soluble fraction of dichloromethane-methanol extract of *Spongia* spp. various

compounds, 12-hydroxyambliofuran (**58**), its acetate (**59**), five tetracyclic diterpenes (**60-64**) and an epoxyfuranosesterterpene carboxylic acid (**65**).[15b]

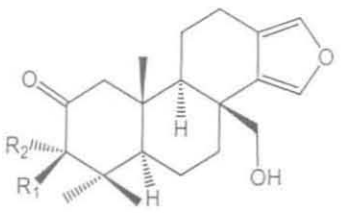


57



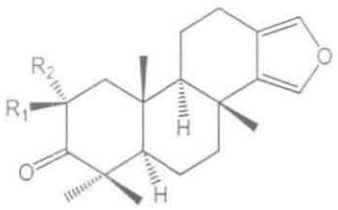
58, $R_1$ = H, $R_2$ = OH

59, $R_1$ = H, $R_2$ = OAc



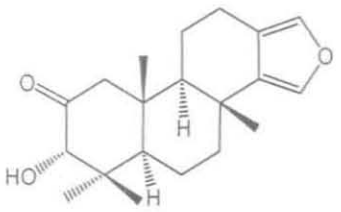
60, $R_1$ = OH, $R_2$ = H

61, $R_1$ = H, $R_2$ = OH

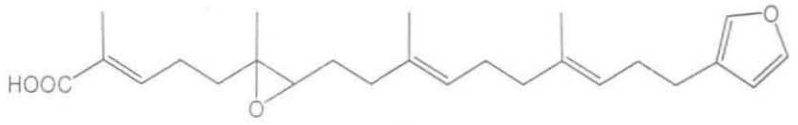


62, $R_1$ = H, $R_2$ = OH

63, $R_1$ = OH, $R_2$ = H

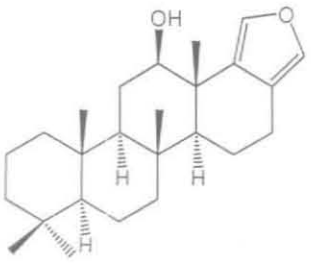


64



65

Recently three new scalarane class sesterterpenoids, 12-*O*-deacetylscalarafuran (**65**), 12-*O*-deacetyl-12-*epi*-scalarin (**66**) and 12-*O*-acetyl-16-*O*-methylhyrtiolide (**67**) were isolated from ethyl acetate soluble portion of methanol extract of *Spongia* spp.[16]



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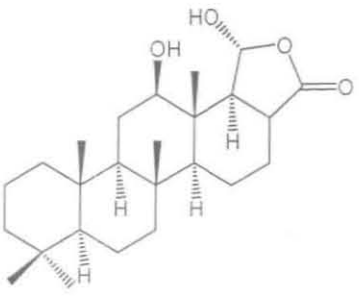
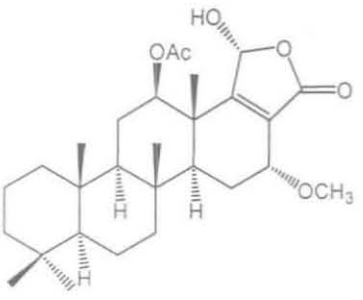


66



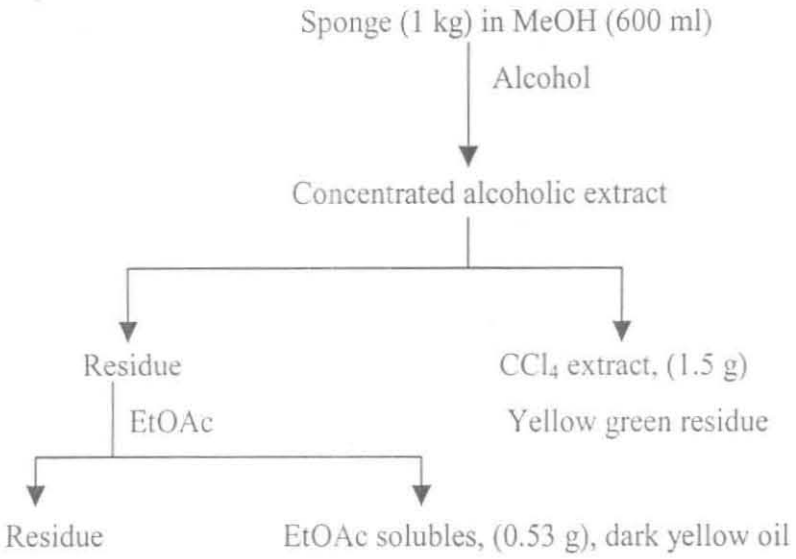
67

**Chemical Analysis of Sponge *Spongia* spp (Fig - 1)**

| | | |
|---|---|---|
| Phylum | - | Porifera |
| Class | - | Demospongia |
| Sub class | - | Ceractinomorpha |
| Order | - | Dictyoceratida |
| Family | - | Spongiidae (Gray 1867) |
| Genus | - | *Spongia* spp.(Linneaus 1759) |

**Fig - 1**

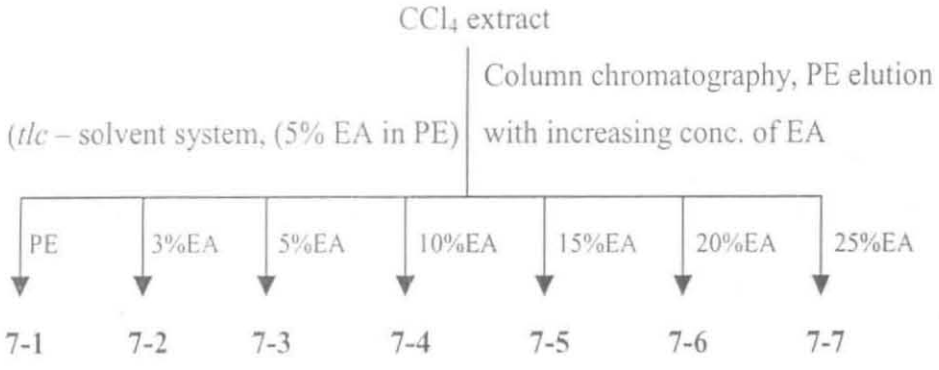
The methanolic extract was obtained by decantation from the initial storage of the sponge *Spongia* spp. (1 kg wet weight) and the sponge was further extracted with ethanol (600 ml). Both alcoholic extracts were combined (~1400 ml) and concentrated. The crude concentrate was extracted with carbon tetracholoride (30 ml three times) and ethyl acetate (250 ml, in portions) to get 1.5 g of yellow green residue and 0.53 g of dark yellow oil respectively. The remaining crude was devoid of any organic solubles as tested by *tlc*. The fractionation is shown in **Scheme - 1**.



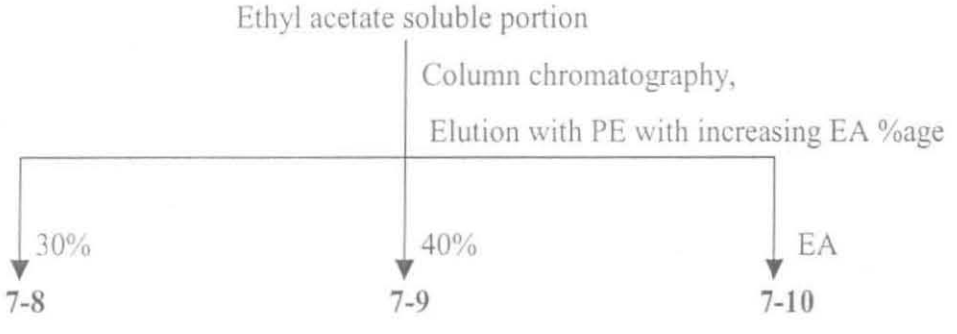

**Scheme - 1**

The carbon tetrachloride extract was column chromatographed over silica gel by spreading the dichloromethane solution of the sample on little silica gel, dried and packed above the column. Pet. ether elution afforded the first fraction, **7-1** (12 mg) as light yellow oil, followed by second fraction, **7-2** (53 mg, light yellow oil) at 3% ethyl acetate elution. Further increase in the concentration of ethyl acetate during elution with petroleum ether gave additional fractions **7-3** (20 mg), **7-4** (111 mg), **7-5** (24 mg), **7-6** (19 mg) and **7-7** (20 mg) **(Scheme – 2).**

Similarly the ethyl acetate extract of the crude was dissolved in pet. ether/ethyl acetate/methanol mixture, spread on little silica gel and dried under air, then loaded to silica gel column in pet. ether. Elution with 80:20 pet. ether – ethyl acetate gave the fraction **7-8**, while with 70:30 pet. ether – ethyl acetate mixture, **7–9** was obtained. Similarly fraction **7-10** was obtained by subsequent increase in concentration of ethyl acetate in the eluting mixture (**Scheme - 3**).




**Scheme - 2**




**Scheme – 3**

Analysis of fraction **7-1 (Table - 1)**

The major component in this fraction was identified as squalene (**7-1-1**) (**Fig - 2**) evidenced by the characteristic molecular fragments in its mass spectrum. The M+1 peak appeared at *m/e* 411 with the base peak at 69 accounting for the isoprenyl unit $C_5H_9$. Isopropyl group fragment at *m/e* 42 as base peak was noticed with moderate intensity. Other prominent fragment units were:  $(C_7H_{11})$ - *m/e* 95;  $(C_8H_{13})$ - *m/e* 109;  $(C_9H_{15})$ - *m/e* 123;  $(C_{10}H_{17})$ - *m/e* 137. In other aspects, the compound shows perfect matching with the standard mass spectrum of squalene (**Table - 1**).

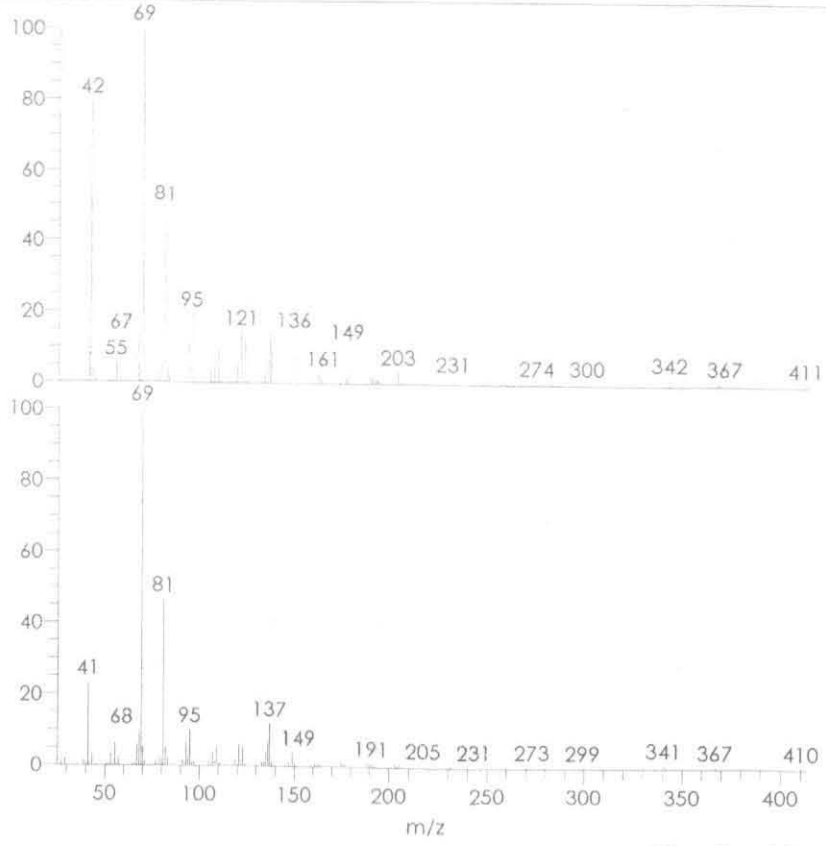
**Table - 1:** Analysis of fraction **7-1**

| Comp No. | RT | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|
| **7-1-1** | 18.97 | $C_{30}H_{50}$ | 410 |  Squalene | 411 | 69, 42, 81, 95, 121, 136, 149 |

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 759 | 782 | 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-I | |
| 2 | 748 | 759 | Squalene | |
| 3 | 732 | 745 | 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-I | |
| 4 | 728 | 736 | Squalene | |
| 5 | 707 | 715 | Squalene | |
| 6 | 685 | 695 | 2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentc | |
| 7 | 661 | 667 | 1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,2 | |
| 8 | 655 | 687 | 2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetrame | |
| 9 | 651 | 662 | Docosa-2,6,10,14,18-pentaen-22-al, 2,6,10,15,18-pe | |
| 10 | 650 | 659 | 6,10,14,18,22-Tetracosapentaen-2-ol, 3-bromo-2,6, | |
| 11 | 647 | 707 | 5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-, (E,I | |
| 12 | 647 | 648 | 2,6,10,15-Tetramethyl-17-(1,4,4-trimethylcyclohex-2 | |
| 13 | 644 | 669 | 2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetrame | |
| 14 | 644 | 645 | 2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadecc | |

Squalene
Formula C30H50, MW 410, CAS# 7683-64-9, Entry# 6377
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-





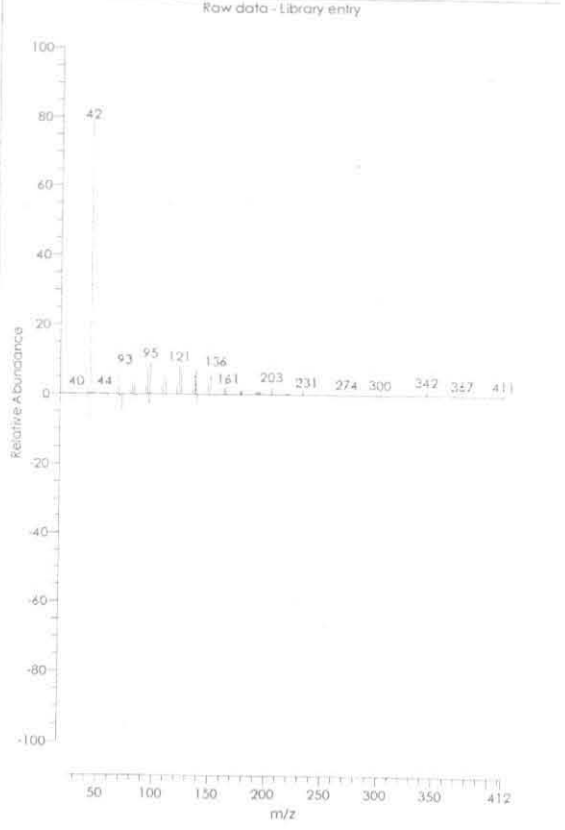




**Fig – 2 Mass spectrum of 7-1-1**

Analysis of fraction **7-2 (Table - 2)**

This fraction contained only methyl esters of long chain fatty acids of both saturated and unsaturated nature as has already been seen previously in least polar fraction of other sponge extracts.

On GC-MS analyses, twenty-three compounds, **7-2-1** to **7-2-23** were identified (**Table - 2**). They are the methyl ester of long chain fatty acids of chain length varying from $C_{14}$ to $C_{24}$ of both saturated and unsaturated chain. Saturated esters show mainly M+1. The mass spectrum is typical of that of hydrocarbons with mass difference of *m/e* 14 accountable for every $-CH_2$ group. The base peak is at *m/e* 74 arising out of the fragment $[H_2C{=}C(OH)OMe]^{+\cdot}$ formed by McLafferty rearrangement. In some instances the base peak appears at *m/e* 42 accounting for propyl fragment. The composition of methyl esters of $C_{15}$ (**7-2-2**), $C_{16}$ (**7-2-3**), $C_{17}$ (**7-2-4**), $C_{18}$ (**7-2-9**) and $C_{19}$ (**7-2-11**) carboxylic acids are well above 10% followed by $C_{23}$ acid (**7-2-20**) of 6.82%. Other esters (**7-2-1**), (**7-2-13**), (**7-2-19**) and (**7-2-23**) are only in trace amounts. **7-2-12** (**Fig - 3**) has a cyclopropyl ring.

Unsaturated esters (**7-2-5**), (**7-2-8**), (**7-2-10**), (**7-2-13**), (**7-2-15**), (**7-2-16**), (**7-2-18**) and (**7-2-22**) (**Fig - 4**) showed the base peak at *m/e* 42 in most cases unlike the other unsaturated esters previously noticed, wherein the base peak appear at *m/e* 55. Rest of mass spectral peak pattern resembles that of straight chain hydrocarbon with mass difference of 14. The fragment with *m/e* 55 of 2-butene unit, cleavage of bond between γ-δ carbons leading to a fragment with *m/e* 87, the fragment with *m/e* 74 as that of saturated esters, *etc.* are some other fragments that can be highlighted.

Analysis of fraction **7-3 (Table - 2)**

Four compounds have been identified in this fraction, three fatty acid methyl esters (**7-3-1**), (**7-3-2**) and (**7-3-3**) and one phthalyl ester (**7-3-4**). The fatty esters as the initial component of the fraction have good matching with the standards.

Compound (**7-3-4**) exhibits mass spectrum similar to that detected in sponge *Hippospongia* sp. and identified to be dioctyl phthalate. The RT value is also almost same as in that case. The details of compounds identified are listed in **Table - 3**.

**Table - 2**: Analysis of fraction **7-2**

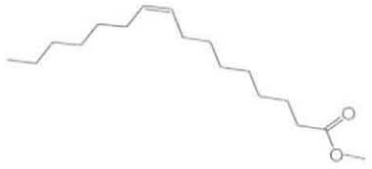
| Comp No. | RT | Peak area %age | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|---|
| **7-2-1** | 8.73 | 0.84 | $C_{15}H_{30}O_2$ | 242 |  Methyl tetradecanoate | 243 | 42, 74, 87, 55, 143, 136, 199 |
| **7-2-2** | 9.44 | 8.15 | $C_{16}H_{32}O_2$ | 256 |  Methyl pentadecanoate | 259 | 74, 87, 43, 55, 199, 213, 143 |
| **7-2-3** | 10.47 | 3.25 | $C_{17}H_{34}O_2$ | 270 |  Methyl hexadecanoate | 272 | 74, 87, 43, 55, 143, 227 |
| **7-2-4** | 11.31 | 7.53 | $C_{18}H_{36}O_2$ | 284 |  Methyl heptadecanoate | 287 | 74, 87, 143, 43, 55, 97, 129, 199, 241 |
| **7-2-5** | 10.63 | 1.58 | $C_{17}H_{32}O_2$ | 268 |  Methyl 9(Z)-hexadecenoate | 268 | 42, 55, 69, 74, 87, 110, 152, 194 |

**Table – 2 contd...**

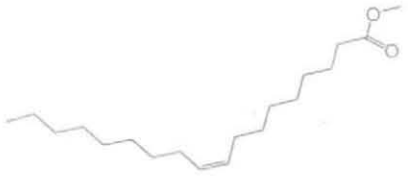
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 7-2-6 | 11.71 | 0.96 | $C_{18}H_{34}O_2$ | 282 |  Methyl 2-hexylcyclopropan octanoate | 252 | 42, 55, 74, 87, 97, 123, 138 |
| 7-2-7 | 9.50 | 0.47 | $C_{16}H_{32}O_2$ | 256 |  Methyl 12-methyl tetradecanoate | 257 | 74, 42, 56, 87, 199, 143, 213, 97 |
| 7-2-8 | 12.61 | 0.87 | $C_{19}H_{36}O_2$ | 296 |  Methyl 9(Z)-octadecenoate | 297 | 55, 42, 74, 83, 97, 264, 222 |
| 7-2-9 | 12.83 | 8.91 | $C_{19}H_{38}O_2$ | 298 |  Methyl octadecanoate | 300 | 74, 87, 43, 55, 143, 255 |

**Table – 2 contd...**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **7-2-10** | 13.17 | 1.68 | $C_{20}H_{38}O_2$ | 310 |  Methyl 10-nonadecenoate | 313 | 42, 69, 74, 55, 87, 97, 279 |
| **7-2-11** | 13.21 | 13.09 | $C_{20}H_{40}O_2$ | 312 |  Methyl nonadecanoate | 313 | 74, 87, 143, 43, 55, 69, 213, 269 |
| **7-2-12** | 13.62 | 5.94 | $C_{20}H_{38}O_2$ | 310 |  Methyl 2-octylcyclopropane octanoate | 311 | 69, 74, 54, 42, 83, 97, 110, 123, 278, 137 |
| **7-2-13** | 14.06 | 0.64 | $C_{21}H_{42}O_2$ | 326 |  Methyl eicosanoate | 327 | 42, 74, 87, 199, 143 |

**Table – 2 contd...**

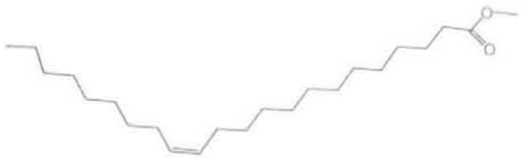
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **7-2-14** | 14.35 | 0.88 | $C_{19}H_{34}O_2$ | 294 |  Methyl 9,12-octadecadienoate | 294 | 42, 67, 55, 81, 95, 109 |
| **7-2-15** | 14.45 | 0.93 | $C_{21}H_{40}O_2$ | 324 |  Methyl 11-eicosenoate | 294 | 42, 55, 69=74, 83, 97, 111 |
| **7-2-16** | 14.21 | 0.42 | $C_{21}H_{38}O_2$ | 322 |  Methyl 8,11-eicosadienoate | 323 | 42, 67~95, 81, 55, 109, 135 |
| **7-2-17** | 15.45 | 0.87 | $C_{22}H_{44}O_2$ | 340 |  Methyl heneicosanoate | 341 | 42, 74, 87, 143, 297 |
| **7-2-18** | 16.08 | 4.98 | $C_{23}H_{44}O_2$ | 352 |  Methyl 13(Z)-docosenoate | 354 | 95, 74, 43, 321, 67, 109, 279, 237 |

**Table – 2 contd...**

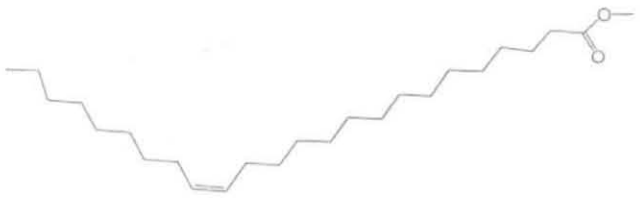
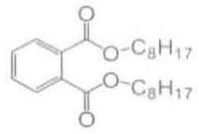
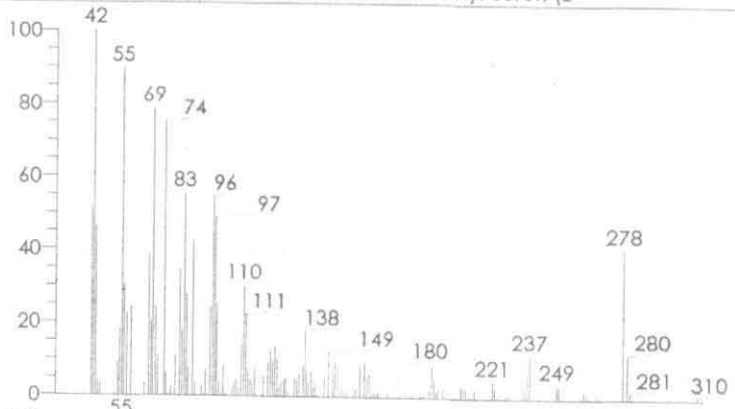
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **7-2-19** | 15.78 | 0.74 | $C_{23}H_{46}O_2$ | 354 |  Methyl docosanoate | 355 | 42, 74, 87, 57, 143, 199, 241, 269, 312 |
| **7-2-20** | 16.60 | 6.82 | $C_{24}H_{48}O_2$ | 368 |  Methyl tricosanoate | 369 | 74, 87, 57, 143, 43, 255, 326 |
| **7-2-21** | 16.98 | 1.10 | $C_{24}H_{46}O_2$ | 366 |  Methyl 2-octylcyclopropane dodecanoate | 367 | 42, 55, 69, 83, 118, 97, 335 |
| **7-2-22** | 17.80 | 1.20 | $C_{25}H_{48}O_2$ | 380 |  Methyl 15(Z)-tetracosenoate | 381 | 42, 349, 55, 69, 74, 83, 97 111, 123, 264, 305 |
| **7-2-23** | 17.93 | 0.49 | $C_{25}H_{50}O_2$ | 382 |  Methyl tetracosanoate | 384 | 42, 74, 87, 143, 97, 199, 340 |

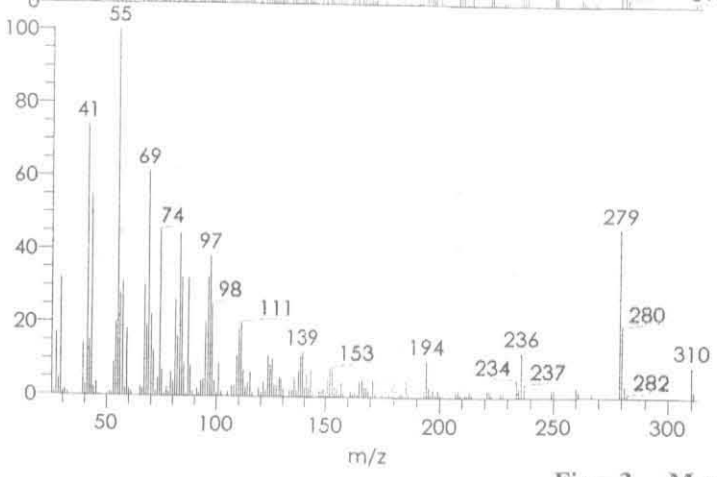
**Table - 3:** Analysis of fraction **7-3**

| Comp No. | RT | Peak area %age | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|---|
| **7-3-1** | 9.38 | 9.99 | $C_{16}H_{32}O_2$ | 256 |  Methyl pentadecanoate | 228 | 74, 87, 55, 69, 143, 213, 115, 185 |
| **7-3-2** | 10.81 | 10.29 | $C_{17}H_{34}O_2$ | 270 |  methyl hexadecanoate | 271 | 74, 87, 55, 143, 97, 129, 171, 227 |
| **7-3-3** | 12.58 | 6.14 | $C_{19}H_{36}O_2$ | 296 |  Methyl 9(Z)-octadecenoate | 268 | 55, 69, 74, 96, 109, 119, 141, 185, 222 |
| **7-3-4** | 16.46 | 42.51 | $C_{24}H_{38}O_4$ | 390 |  Dioctyl phthalate | 285 | 149, 167, 57, 71, 104 |

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 14 | 691 | 848 | 9-Hexadecenoic acid, methyl ester, (Z | |
| 15 | 686 | 700 | Decanoic acid, 10-(2-hexylcycloprop | |
| 16 | 684 | 770 | 9-Octadecenoic acid (Z)-, methyl est | |
| 17 | 684 | 831 | 13-Docosenoic acid, methyl ester, (Z) | |
| 18 | 682 | 697 | 9-Octadecenoic acid, methyl ester, ( | |
| 19 | 680 | 722 | Cyclopropaneoctanoic acid, 2-octyl | |
| 20 | 679 | 685 | 13-Docosenoic acid, methyl ester, (Z) | |
| 21 | 679 | 740 | 9-Hexadecenoic acid, methyl ester, (Z | |
| 22 | 678 | 693 | 11-Octadecenoic acid, methyl ester, | |
| 23 | 671 | 673 | 9-Octadecenoic acid, methyl ester | |
| 24 | 670 | 690 | 9-Octadecenoic acid (Z)-, methyl est | |
| 25 | 670 | 759 | 9-Octadecenoic acid (Z)-, methyl est | |
| 26 | 667 | 761 | Cyclopropaneoctanoic acid, 2-hexyl | |
| 27 | 667 | 738 | 7-Hexadecenoic acid, methyl ester, (Z | |




5-5#1521 RT: 13.61 AV: 1 NL: 1.73E5 T: {0.0} + c EI det=350.00 Full ms [ 40.00-600.00]




SI 680, RSI 722, MAINLIB, Entry# 13253, CAS# 5135-07-9, Cyclopropaneoctanoic acid, 2-octyl-, methyl ester, trans-

Cyclopropaneoctanoic acid, 2-octyl-, methyl ester, trans-
Formula C20H38O2, MW 310, CAS# 5135-07-9, Entry# 13253

Raw data - Library entry

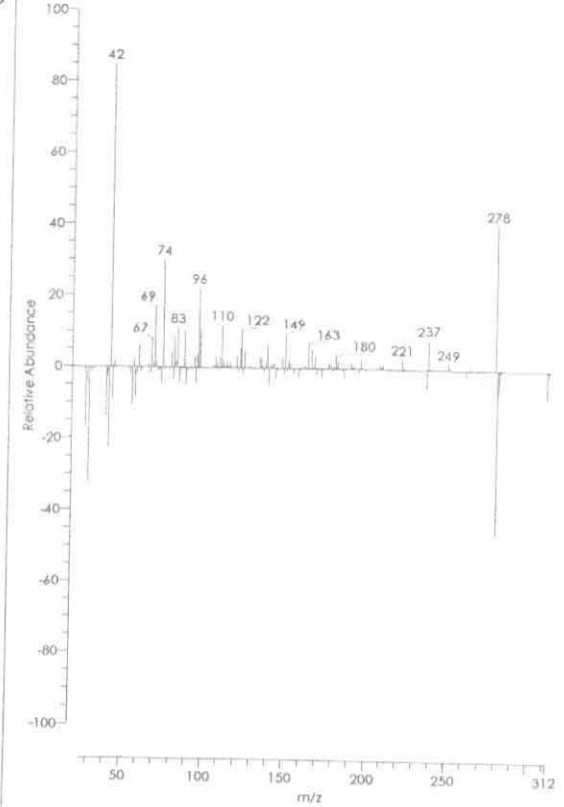
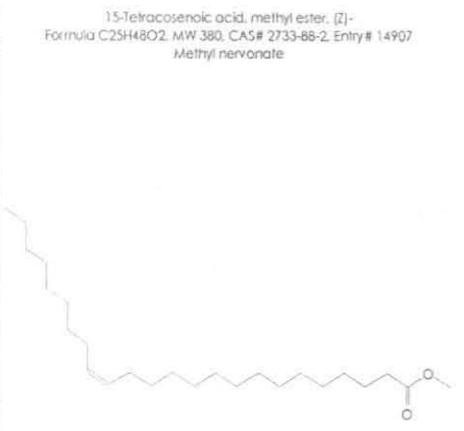
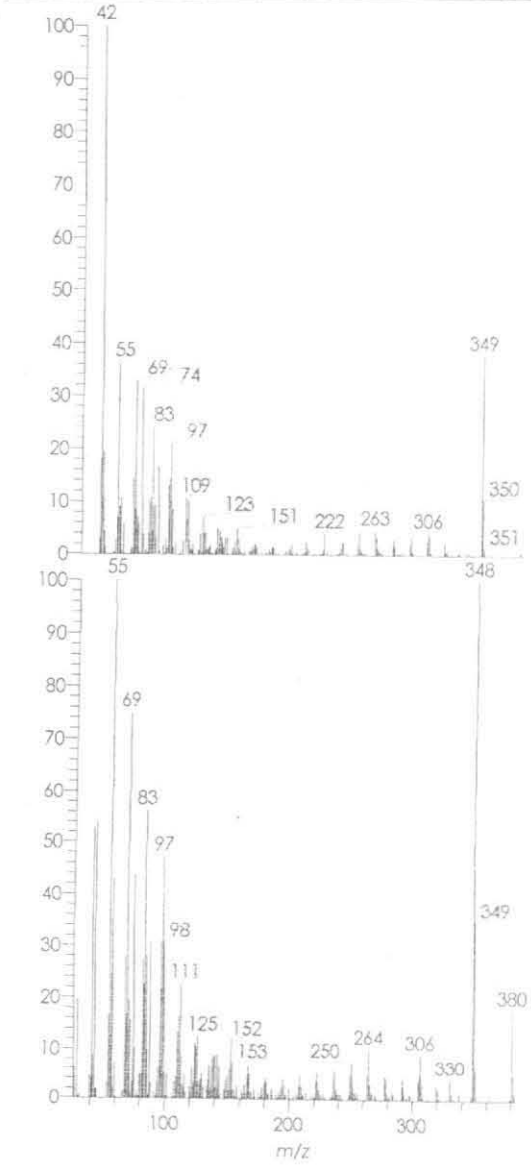



**Fig – 3 Mass spectrum of 7-2-12**

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 684 | 686 | 15-Tetracosenoic acic | |
| 2 | 676 | 676 | 15-Tetracosenoic acic | |
| 3 | 667 | 693 | 15-Tetracosenoic acic | |
| 4 | 644 | 717 | 13-Docosenoic acid, m | |
| 5 | 630 | 819 | Triolein | |
| 6 | 630 | 816 | 9-Octadecenoic acic | |
| 7 | 627 | 790 | 9-Octadecenoic acic | |
| 8 | 627 | 790 | 9-Octadecenoic acic | |
| 9 | 614 | 795 | 9-Octadecenoic acic | |
| 10 | 612 | 795 | 9-Octadecenoic acic | |
| 11 | 606 | 747 | 9-Octadecenoic acic | |
| 12 | 603 | 774 | 9-Octadecenoic acic | |
| 13 | 601 | 734 | 11-Octadecenoic aci | |
| 14 | 596 | 725 | 9-Octadecenoic acic | |
| 15 | 595 | 769 | 9-Hexadecenoic acic | |
| 16 | 591 | 737 | 11-Octadecenoic aci | |
| 17 | 591 | 725 | Cyclopropanedecanc | |
| 18 | 589 | 716 | Cyclopropaneoctanc | |



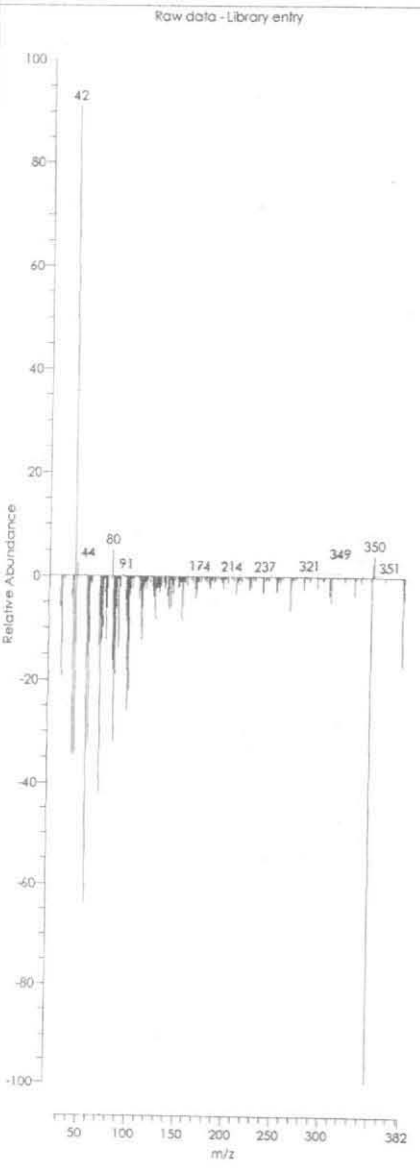




Fig – 4 Mass spectrum of 7-2-22

Compound (**7-3-4**) has appreciable composition ~ 43%, while (**7-3-1**), (**7-3-2**) and (**7-3-3**) are about or less than 10%.

Analysis of fraction **7-4 (Table – 4)**

Being a higher polar fraction, this fraction is having six free fatty acids and two steroidal derivatives. Thus eight compounds have been identified. The chain length in carboxylic acids (**7-4-1** to **7-4-6**) varies from $C_{15}$ to $C_{19}$. They have base peak mostly at *m/e* 73 for saturated acids and at 55 for unsaturated acids. The typical elimination of acetic acid with *m/e* 60 is evident in all the mass spectra of the carboxylic acids. The fragment ion $[CH_2CH_2COOH]^{+\cdot}$ from $\beta$-$\gamma$ bond cleavage accounting for the fragment at *m/e* 73 is also prevalent. Other intermittent peak clusters with regular mass difference of 14 is typical of straight chain nature of the acids. The other spectral details are given in **Table - 4**. **7-4-7** is identified as cholesterol and **7-4-8** as cholest-7-en-3-ol.

Analysis of fraction **7-5 (Table – 5)**

This fraction has three carboxylic acids **7-5-1** to **7-5-3** as detailed in **Table - 5**.

Analysis of fraction **7-6 (Table – 6)**

This fraction has a carboxylic acid **7-6-1** and an ester **7-6-2** as detailed in **Table - 6**.

Analysis of fraction **7-7(Table – 7)**

This fraction contains a single compound, **7-7-1** (**Fig - 5**) and that is identified as methyl salicylate. The following flow chart shows the possible fragment pattern. Details are presented in **Table - 7**.

**Table - 4:** Analysis of fraction **7-4**

| Comp No. | RT | Peak Area %age | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|---|
| **7-4-1** | 10.26 | 0.12 | $C_{15}H_{30}O_2$ | 242 |  Pentadecanoic acid | 242 | 73, 55, 83, 129, 97, 199, 111, 185 |
| **7-4-2** | 11.17 | 2.05 | $C_{16}H_{32}O_2$ | 256 |  Hexadecanoic acid | 256 | 73, 60, 55, 129, 83, 96, 157, 213, 171 |
| **7-4-3** | 11.97 | 4.18 | $C_{17}H_{34}O_2$ | 270 |  Heptadecanoic acid | 271 | 55, 69, 73, 83, 97, 129, 115, 152, 171, 185 |
| **7-4-4** | 13.24 | 4.08 | $C_{18}H_{34}O_2$ | 282 |  9(E)- Octadecenoic acid | 285 | 55, 69, 83, 97 111, 129, 152, 185, 264 |
| **7-4-5** | 13.41 | 5.35 | $C_{18}H_{36}O_2$ | 284 |  Octadecanoic acid | 285 | 73, 55, 83, 129, 96, 115, 185, 171, 242, 199 |

**Table – 4 Contd...**

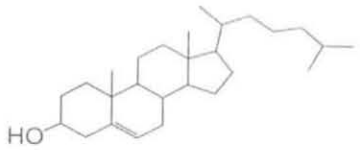
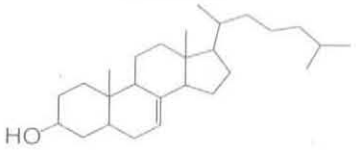
| Comp No. | RT | Peak Area %age | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|---|
| **7-4-6** | 13.77 | 9.32 | $C_{19}H_{38}O_2$ | 298 |  Nonadecanoic acid | 300 | 55, 69, 83, 97, 129, 111, 137, 199 |
| **7-4-7** | 24.06 | 22.77 | $C_{27}H_{46}O$ | 386 |  Cholesterol | 387 | 145, 107, 81, 119, 55, 213, 354, 255, 275, 276, 327 |
| **7-4-8** | 25.25 | 3.68 | $C_{27}H_{46}O$ | 386 |  Cholest-7-en-3-ol | 388 | 55, 95, 105, 255, 133, 159, 213, 229 |

**Table - 5:** Analysis of fraction **7-5**

| Comp No. | RT | Peak Area %age | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|---|
| **7-5-1** | 9.50 | 2.33 | $C_{14}H_{28}O_2$ | 228 |  Tetradecanoic acid | 228 | 73, 55, 60, 83, 129, 98, 115, 185, 171 |
| **7-5-2** | 10.17 | 18.37 | $C_{15}H_{30}O_2$ | 242 |  Pentadecanoic acid | 242 | 73, 60, 83, 129, 97, 199, 143, 185, 157 |
| **7-5-3** | 11.91 | 8.42 | $C_{16}H_{32}O_2$ | 256 |  Hexadecanoic acid | 256 | 69, 55, 83, 97, 111, 129, 152, 185, 213 |

**Table - 6:** Analysis of fraction **7-6**

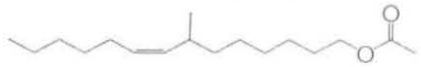
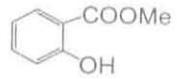
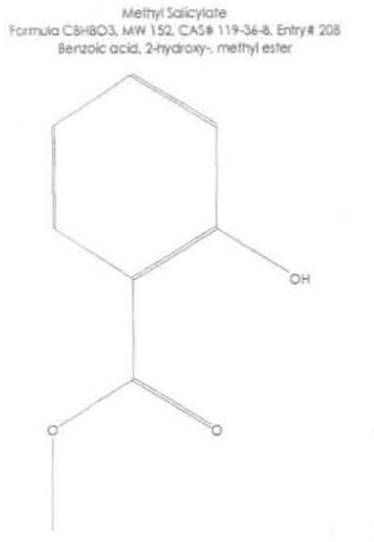
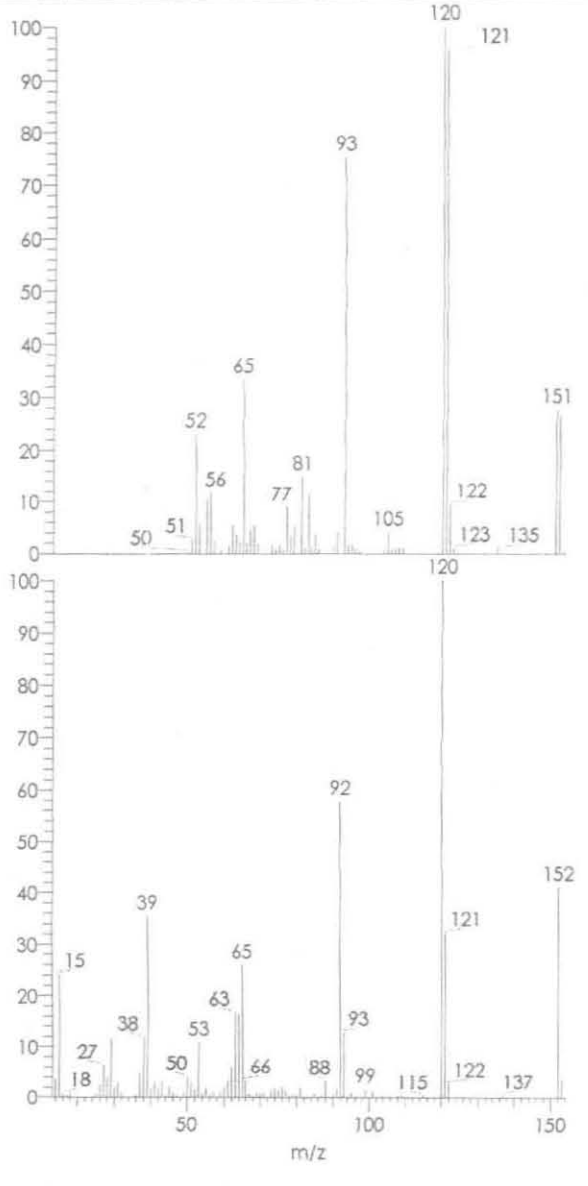
| Comp No. | RT | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|
| **7-6-1** | 11.55 | $C_{16}H_{32}O_2$ | 256 |  Hexadecanoic acid | 258 | 73, 60, 83, 129, 97, 115, 157, 171, 213 |
| **7-6-2** | 11.92 | $C_{17}H_{32}O_2$ | 268 |  7-Methyl tetradec-8-en-1-yl acetate | 268 | 69, 55, 83, 97, 111, 152, 171 |

**Table - 7:** Analysis of fraction **7-7**

| Comp No. | RT | Peak Area %age | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|---|
| **7-7-1** | 8.62 | 6.69 | $C_8H_8O_3$ | 152 |   Methyl salicylate | 152 | 120, 93, 65, 52, 81 |

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 610 | 626 | Benzoic acid, 4-amino-, methyl ester | |
| 2 | 609 | 614 | Methyl Salicylate | |
| 3 | 598 | 655 | Methyl Salicylate | |
| 4 | 597 | 597 | Benzoic amide, 2-hydroxy-N-methyl- | |
| 5 | 596 | 699 | Pyridine, 2-ethyl-6-methyl- | |
| 6 | 592 | 623 | Methylparaben | |
| 7 | 590 | 639 | Methyl Salicylate | |
| 8 | 590 | 639 | Methyl Salicylate | |
| 9 | 590 | 595 | Salicylamide, N-methyl- | |
| 10 | 584 | 604 | Benzoic acid, 3-hydroxy-, methyl ester | |
| 11 | 581 | 695 | Benzenamine, N,3-dimethyl- | |
| 12 | 577 | 652 | 2-Amino-4-methylpyrrole-3-carbonitrile | |
| 13 | 576 | 585 | Urocanic acid, methyl ester | |
| 14 | 572 | 715 | Pyridine, 2-ethyl-6-methyl- | |
| 15 | 572 | 609 | Benzoic acid, 4-amino-, methyl ester | |
| 16 | 572 | 685 | 3-Pyridinecarboxylic acid, 4-amino-, methyl ( | |
| 17 | 571 | 636 | Urocanic acid, methyl ester | |
| 18 | 571 | 601 | Methylparaben | |



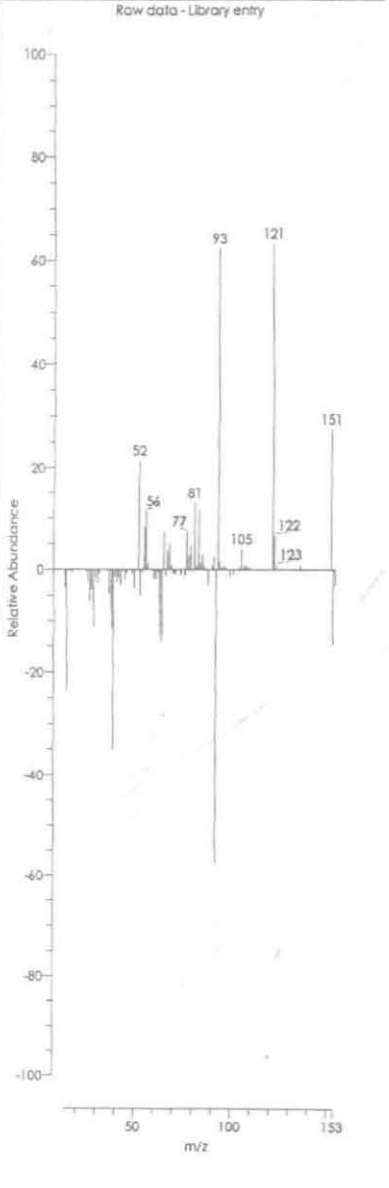
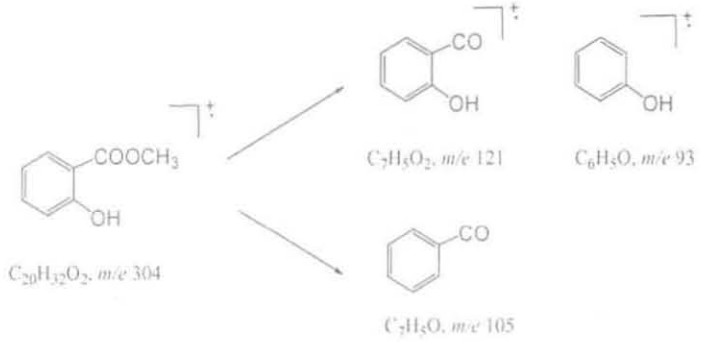




**Fig – 5 Mass spectrum of 7-7-1**




Analysis of fraction **7-8** (**Table - 8**)

This fraction contains two compounds **7-8-1** (**Fig - 6**) and **7-8-2** (**Table - 8**) both being phenolic derivatives. **7-8-1** is shown to be *p*-hydroxybenzaldehyde and the fraction at *m/e* 93 and 65 can be accounted by $[C_6H_5O]^{+\cdot}$ and $[C_6H_5]^{+\cdot}$ fragments. The second compound **7-8-2** has the same mass as that of **6-8-1** and it is shown to be 3,4-dimethylphenol by comparision with mass library. The peak at *m/e* 107 can be accounted by the fragment $[C_7H_7O]^{+\cdot}$

Analysis of fraction **7-9** (**Table - 9**)

This fraction also contains aromatic compounds in minor amounts. The compounds identified are benzoic acid (**7-9-1**, **Fig - 7**), phenylacetic acid (**7-9-2**), 1,4-dimethoxybenzene (**7-9-3**, **Fig - 8**) and dioctyl phthalate (**7-9-4**) (**Table - 9**)

Analysis of fraction **7-10** (**Table - 10**)

This final fraction has been found to have 3-hydroxybenzeneacetic acid (**7-10-1**) (**Fig - 9**) as revealed by a comparision with mass spectral library (**Table - 10**).

**Table - 8**: Analysis of fraction **7-8**

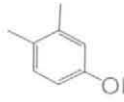
| Comp No. | RT | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|
| **7-8-1** | 6.46 | $C_7H_6O_2$ | 122 |  4-Hydroxybenzaldehyde | 123 | 42, 121, 65, 93 |
| **7-8-2** | 7.06 | $C_8H_{10}O$ | 122 |  3,4-Dimethylphenol | 122 | 42, 107, 65, 77 |

**Table - 9: Analysis of fraction 7-9**

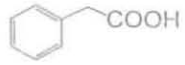
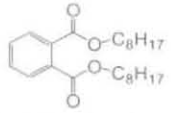
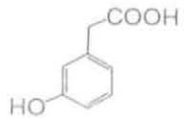
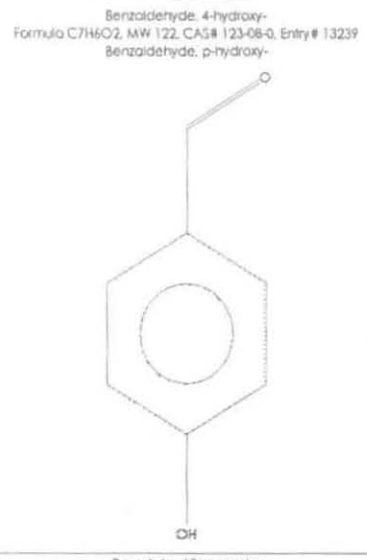
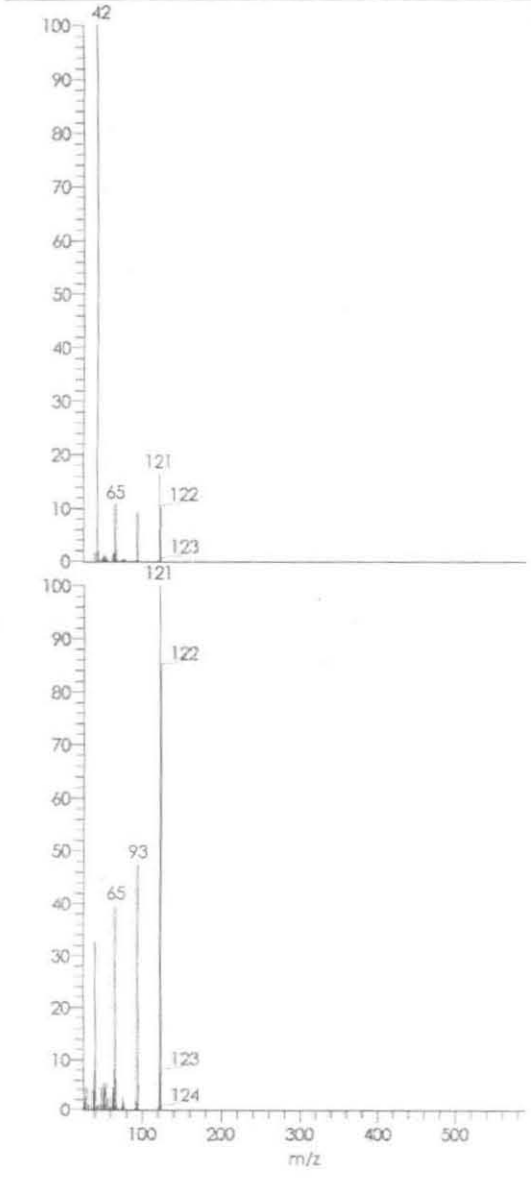
| Comp No. | RT | Peak area %age | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|---|
| **7-9-1** | 3.40 | 3.48 | $C_7H_6O_2$ | 122 |  Benzoic acid | 123 | 105, 101, 77, 55 |
| **7-9-2** | 4.44 | 9.36 | $C_8H_8O_2$ | 136 |  Bezeneacetic acid | 138 | 91, 65 |
| **7-9-3** | 9.15 | 25.65 | $C_8H_{10}O_2$ | 138 |  1,4-Dimethoxybenzene | 139 | 123, 93, 77, 65 |
| **6-9-4** | 16.46 | 4.23 | $C_{24}H_{38}O_4$ | 390 |  Dioctyl phthalate | 281 | 149, 167, 57, 71, 83, 117 |

**Table - 10**: Analysis of fraction **7-10**

| Comp No. | RT | MF | MW | Structure | 1st MS Peak | Prominent peaks (1st value = BP) |
|---|---|---|---|---|---|---|
| **7-10-1** | 10.46 | $C_8H_8O_3$ | 152 |  3-Hydroxybenzeneacetic acid | 152 | 107, 77, 52, 121, 93, 138 |

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 763 | 788 | Propanoic acid, 3-chloro-, 4-formylph | |
| 2 | 648 | 654 | Benzaldehyde, 4-hydroxy- | |
| 3 | 648 | 654 | Benzaldehyde, 4-hydroxy- | |
| 4 | 637 | 638 | Benzaldehyde, 4-hydroxy- | |
| 5 | 623 | 623 | Benzaldehyde, 4-hydroxy- | |
| 6 | 616 | 617 | Benzaldehyde, 3-hydroxy- | |
| 7 | 607 | 609 | Benzaldehyde, 3-hydroxy- | |
| 8 | 581 | 581 | Coumaran-3-one, 6-methoxy-2-[4-h | |
| 9 | 571 | 572 | Benzaldehyde, 2-hydroxy- | |
| 10 | 571 | 572 | Benzaldehyde, 2-hydroxy- | |
| 11 | 569 | 573 | Benzaldehyde, 2-hydroxy- | |
| 12 | 563 | 563 | Benzaldehyde, 2-hydroxy- | |
| 13 | 560 | 561 | Benzaldehyde, 2-hydroxy- | |
| 14 | 556 | 703 | Benzeneethanamine, 4-methoxy- | |
| 15 | 550 | 552 | 1,3-Benzodioxole | |
| 16 | 547 | 553 | 1,3-Benzodioxole | |
| 17 | 544 | 717 | 4-Acetoxybenzaldehyde | |
| 18 | 541 | 543 | Formamide, N-phenyl- | |



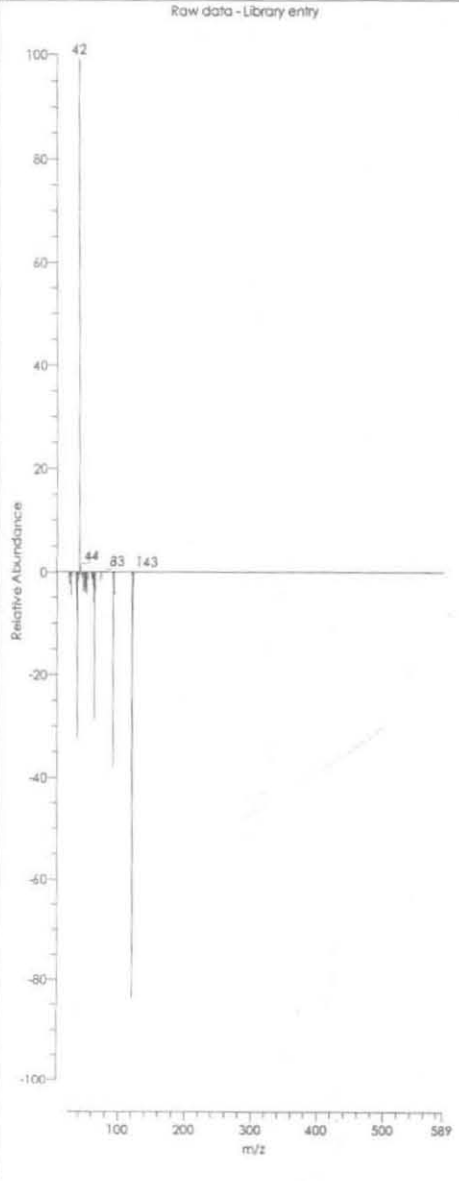
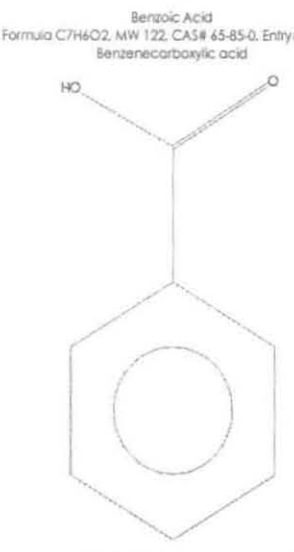
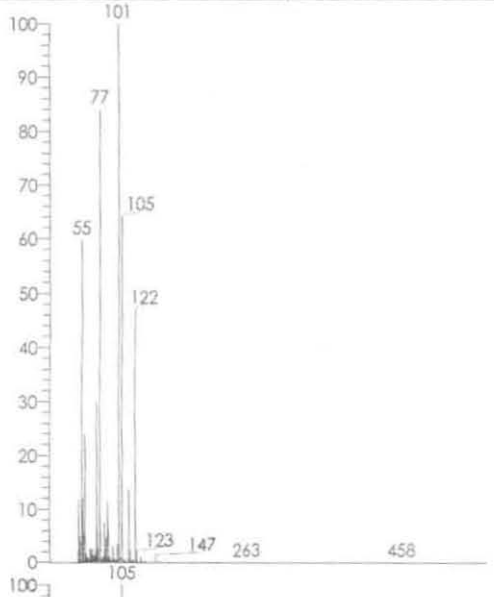



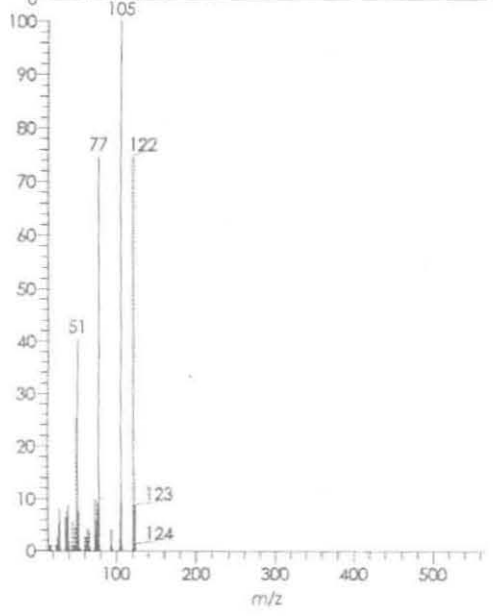

Fig – 6 Mass spectrum of 7-8-1

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 573 | 573 | Glucosamine, N-acetyl | |
| 2 | 564 | 719 | Benzoic Acid | |
| 3 | 558 | 750 | Benzoic Acid | |
| 4 | 554 | 608 | Butanedioic acid, mono | |
| 5 | 545 | 718 | Benzoic Acid | |
| 6 | 545 | 718 | Benzoic Acid | |
| 7 | 533 | 534 | (d)-(+)-(2R,3R)-2,3-Dibe | |
| 8 | 527 | 699 | Benzoic acid, hydrazide | |
| 9 | 523 | 765 | Ethanone, 1,2-diphenyl | |
| 10 | 518 | 532 | 2,3-Di-O-benzoyl-d-ribc | |
| 11 | 514 | 784 | Benzoic Acid | |
| 12 | 503 | 503 | 4-Amino-1,5-pentandic | |
| 13 | 494 | 512 | Monoacetyl-dibenzoyl- | |
| 14 | 492 | 503 | Butanedioic acid, 2,3-b | |
| 15 | 492 | 640 | β-1,5-Dibenzoyl-ribofurc | |
| 16 | 491 | 492 | 2-Phenyl-4,5-[5,6-O-isop | |
| 17 | 488 | 492 | β-1,5-Dibenzoyl-2-deox | |
| 18 | 480 | 500 | 1,3-Dioxolane, 2,2-dime | |




5-7-2#343 RT: 3.40 AV: 1 NL: 1.13E5 T: {0.0} + c EI det=350.00 Full ms [ 50.00-600.00]




SI 558, RSI 750, REPLIB, Entry# 11239, CAS# 65-85-0, Benzoic Acid

Raw data - Library entry

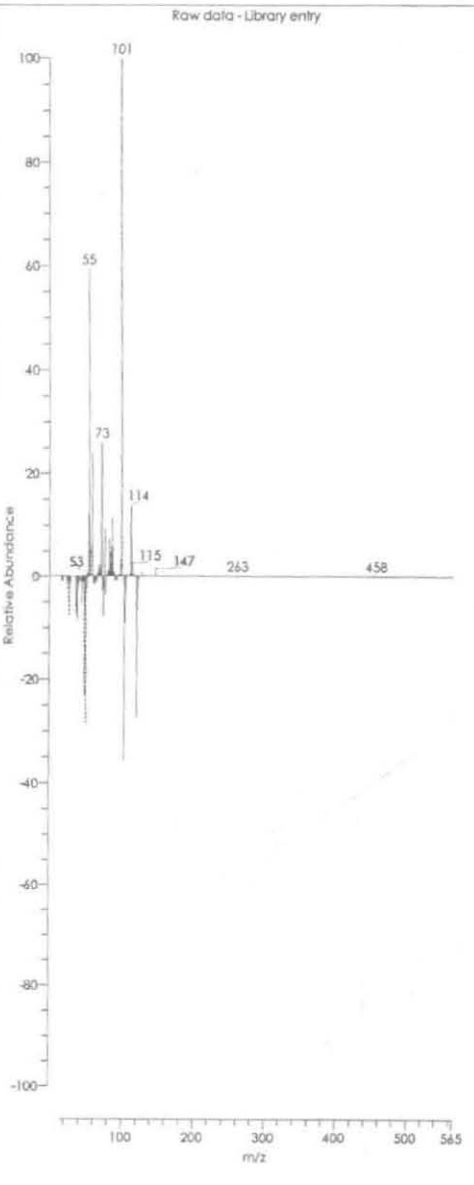
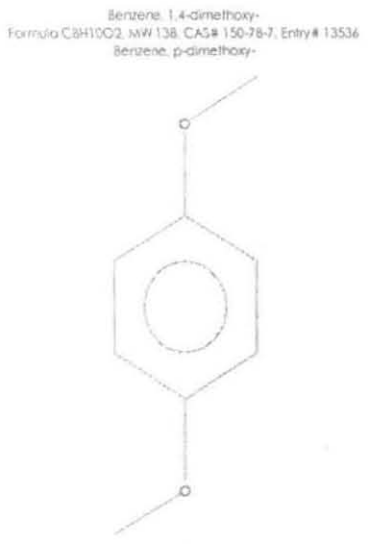
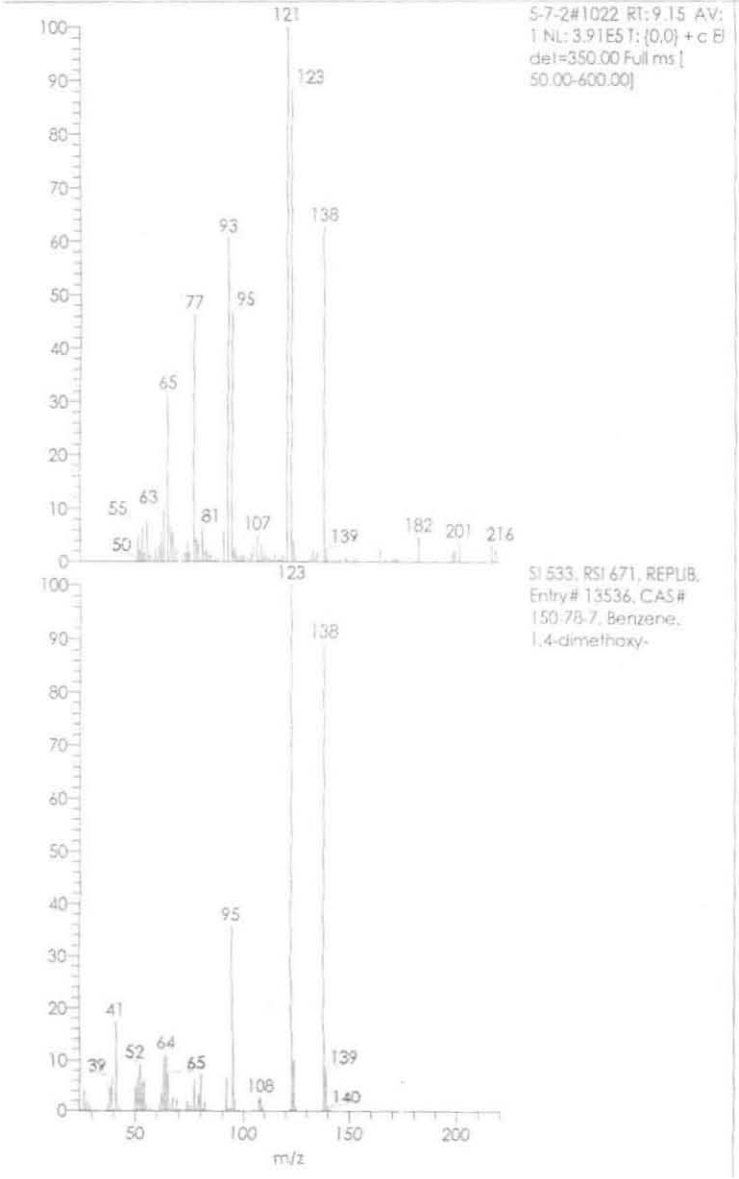



Fig – 7 Mass spectrum of 7-9-1

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 57 | 537 | 582 | Cyclohexene, 1-methy | |
| 58 | 536 | 570 | 4-Acetyl-1-methylcyclc | |
| 59 | 536 | 746 | 1-Bromo-3,3,3-trifluoroc | |
| 60 | 536 | 639 | Propanoic acid, 3-bror | |
| 61 | 535 | 661 | Cyclohexene, 4-methy | |
| 62 | 535 | 558 | 3-Bromomethyl-3,6,6-tr | |
| 63 | 534 | 566 | Benzene, 1-methyl-4-(r | |
| 64 | 534 | 588 | Phenol, 2-methoxy-3-r | |
| 65 | 533 | 671 | Benzene, 1,4-dimethox | |
| 66 | 533 | 559 | 3,4,4-Trimethyl-3-(3-oxc | |
| 67 | 532 | 676 | Benzene, 1,4-dimethox | |
| 68 | 531 | 584 | ±-4-Acetyl-1-methylcyc | |
| 69 | 531 | 694 | Benzeneacetic acid, α | |
| 70 | 528 | 679 | Phenol, 4-methoxy-3-r | |
| 71 | 526 | 684 | Benzene, 1,4-dimethox | |
| 72 | 525 | 686 | Benzene, 1,4-dimethox | |
| 73 | 525 | 686 | Benzene, 1,4-dimethox | |
| 74 | 523 | 557 | Nona-3,5-dien-2-one | |



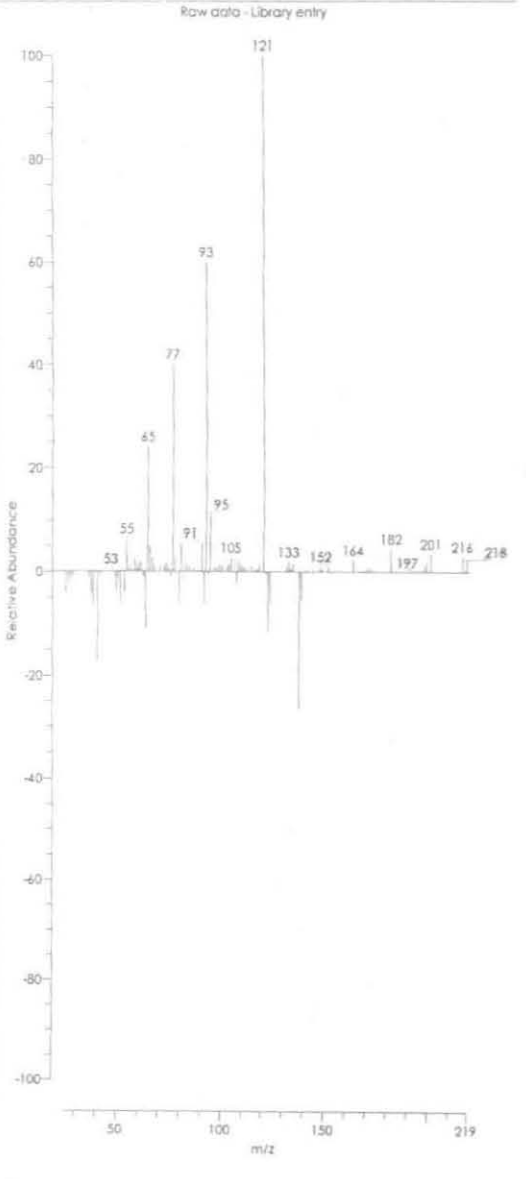
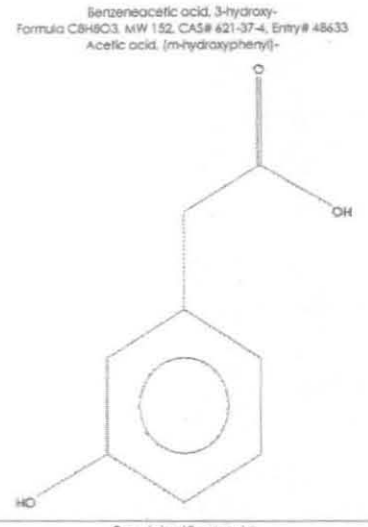
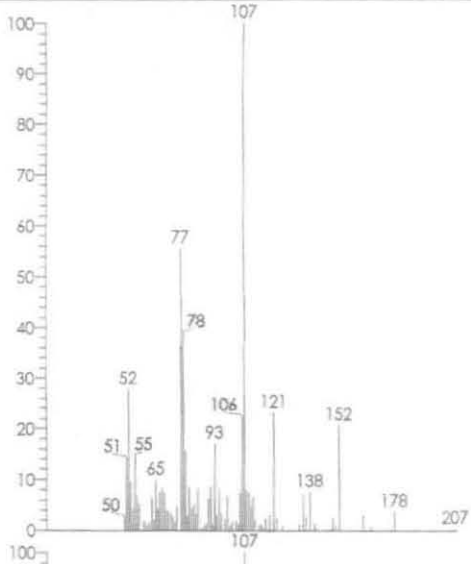
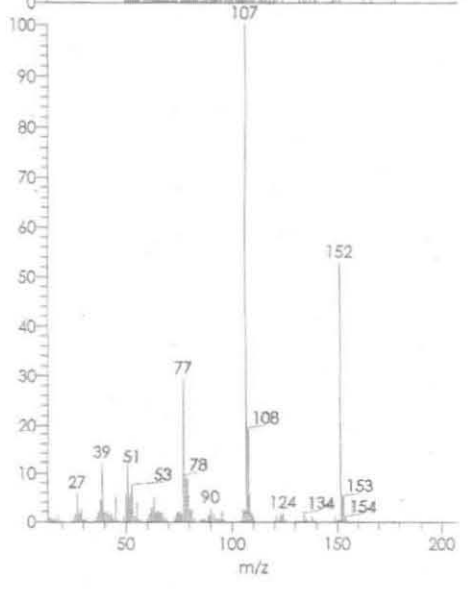




Fig – 8 Mass spectrum of 7-9-3

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 659 | 672 | 3-Hydroxy-4-methylbenzoic acid | |
| 2 | 645 | 685 | Benzeneacetic acid, 3-hydroxy- | |
| 3 | 630 | 681 | Benzeneacetic acid, α-hydroxy- | |
| 4 | 630 | 709 | Bicyclo[3.1.1]hept-2-ene-2-carb | |
| 5 | 623 | 695 | Bicyclo[3.1.1]hept-2-ene-2-carb | |
| 6 | 616 | 643 | 3-Caren-10-al | |
| 7 | 616 | 641 | 1-Oxaspiro[3.5]nonan-2-one, 3-m | |
| 8 | 614 | 690 | Bicyclo[3.1.1]hept-2-ene-2-carb | |
| 9 | 607 | 662 | Benzeneacetic acid, 4-hydroxy- | |
| 10 | 604 | 627 | Bicyclo[3.1.1]hept-2-ene-2-meth | |
| 11 | 601 | 651 | 3a,6-Methano-3ah-inden-4-ol, c | |
| 12 | 600 | 638 | 4-Oxatetracyclo[5.2.2.0(1.6).0(3, | |
| 13 | 599 | 615 | Methanesulfonic acid, 2-(2-hydr | |
| 14 | 598 | 627 | 1-Cyclohexene-1-methanol, 4-(1 | |
| 15 | 596 | 626 | Bicyclo[3.1.1]hept-2-ene-2-meth | |
| 16 | 594 | 662 | Salicyl Alcohol | |
| 17 | 592 | 640 | Bicyclo[3.1.1]hept-2-ene-2-meth | |
| 18 | 589 | 603 | 2(5H)-Furanone, 4-methyl-3-(2-m | |



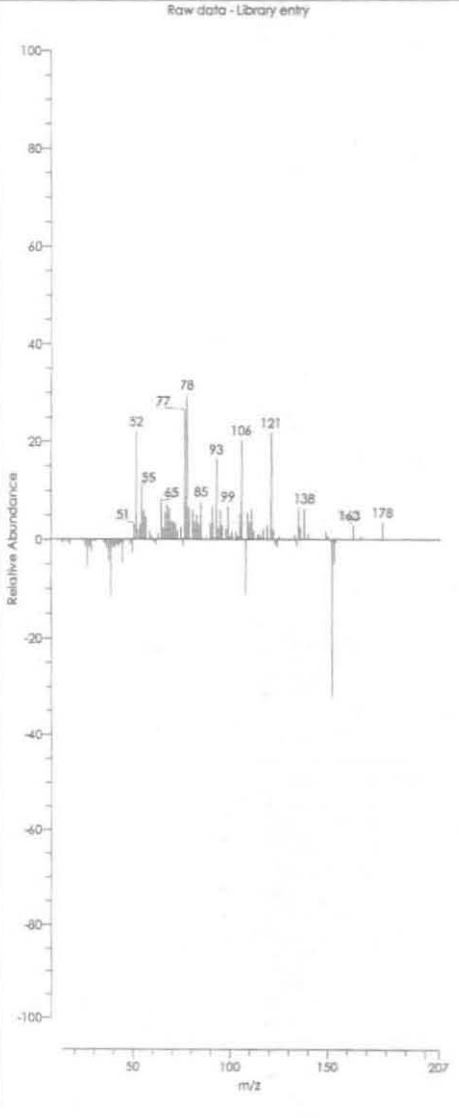




**Fig – 9 Mass spectrum of 7-10-1**

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Chapter 8

# Chemical Investigation of puffer fish, *Tetraodon* spp.

**Bioactive compounds from marine fin fishes**

The bioactive compounds that have been isolated and characterized from marine fin fishes acting as defensive chemicals are called marine toxins or ichthyotoxins.[1] They are found in two forms, as phenero toxic and crypto toxic; the former having the venomous apparatus and the latter having the toxin incorporated in body tissues gained by food chain, symbiotic parasites and biosynthetic transformations of the acquired chemicals. Earliest records of sea food poisoning came from Egyptians dating back to 1600 B.C. as recorded by Pliny the Elder in *Historia-Naturalis*.[2] Much concern about this study was stimulated by the outbreak of sea food poisoning on consumption of food fishes. The other types of compounds from the marine organisms have been separately dealt under marine natural products as reviewed in Chapter 1. According to the type of body part in which toxins occur, they are classified as ichthyosarcotoxic fishes (musculature, viscera or skin eg. ciguatera), ichtyootoxic fishes (gonads) and ichtyohemotoxic fishes (blood eg. moray eel). Many of the toxins are isolated from lyophilic extracts unlike the major organic compounds isolated in lyophobic mode from marine resources.

The toxins derived from dinoflagellates[3] (Protistans, unicellular primary organisms) like *Gonyaulax catenella, G. tamarensis, Gambierdiscus toxicus, Gymnodinium breve, Prorocentrum concavum, Ostreopsis siamensis* (benthic dinoflagellates) are responsible for the toxic accumulation among sea organisms. These toxins contribute to the major outbreak of seasonal food poisoning. They form the red bloom and cause the large-scale fish mortality by asphyxiation. With their ability to photosynthesize, they are the basis of marine food chain.

The food-chain relationship lies as:

Benthic organism → Herbivorous fish → Carnivorous fish

The toxicity grade of the fish body part is in the order of:

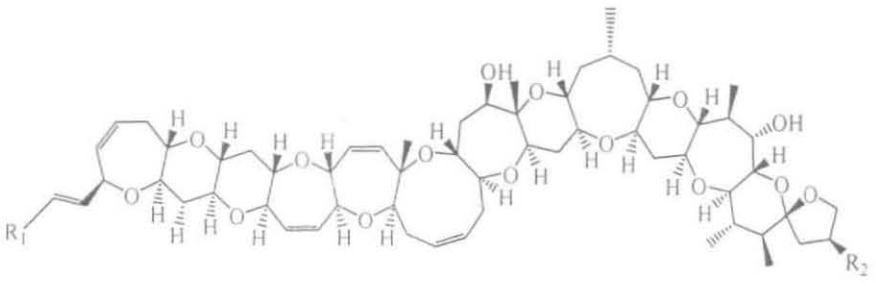
Testes/Ovary (gonads) = Liver > Viscera > Flesh

Some of the major fish toxins are discussed below.

**Ciguatera toxin**

It is lipid soluble and heat resistant. Its analogues are CTX-1, CTX-2, CTX-3 and CTX-4B. It was first recognised by the ingestion of marine snail *Livona pica* in Caribbean.[4] More than 300 species of tropical region fishes[5] like, barracudas, groupers, sea basses, snappers, surgeon fishes, parrot fishes, moray eels and gastropods are the carriers of this toxin. Ciguatera fish poisoning is associated with epiphytic dinoflagellate toxins accumulated in the flesh of fishes acquired through food web from clams, mussels, oysters and scallops, which are the filter feeders. The word '*cigua*' is a Spanish trivial name for univalve mollusk, *Turbo pica*. The dinoflagellates responsible for this toxin are *Gambierdiscus toxicus, Amphidinium carterae, Coolia monotis, Prorocentrum* spp.,*Ostreopsis* spp. *and Thecadinum* spp. Ciguatera is the phenomena of sea food poison out break involving this toxin by ingestion of coral reef fishes that have become toxic through diet. It has effects of gastrointestinal and neurological disorders. The toxins involved in ciguateric poisoning are of two groups; one is ciguatoxin (CTX, **1**) and its congeners (CTX-4B, **2**) and the other being maitotoxin (MTX, **3**). Its structural feature is a ladder like skeleton consisting of trans-fused polyether rings. Dozens of ciguatoxin analogs have been found in fish but only a few of them have been characterized structurally.[6]

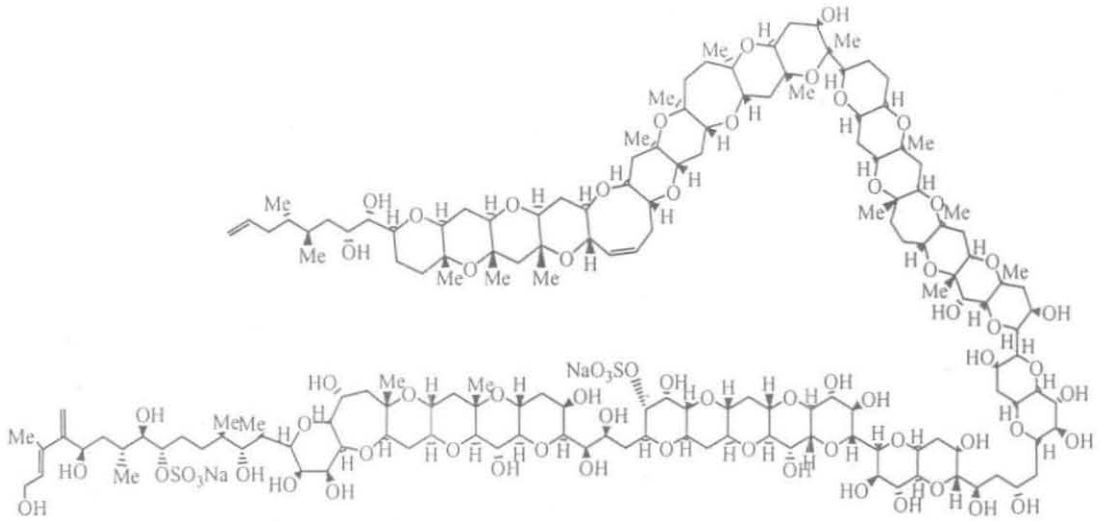
The molecular formula[7] of **1** is $C_{60}H_{86}O_{19}$, with estimated molecular weight of 1111.5843



1, $R_1$ = -CH(OH)-$CH_2$OH; $R_2$ = OH

2, $R_1$ = -CH=$CH_2$; $R_2$ = H

Toxin **3** has the molecular formula of $C_{164}H_{256}O_{68}S_2Na_2$, constructed from a $C_{142}$ carbon chain containing 32 ether rings, 28 hydroxyl groups, and two sulphate ester functions with a molecular weight of 3422 Dalton as its sodium salt. It is one of the most potent, non-proteineceous toxins with regard to its lethality. The total structure was proposed based on the spectroscopic analysis.[8]



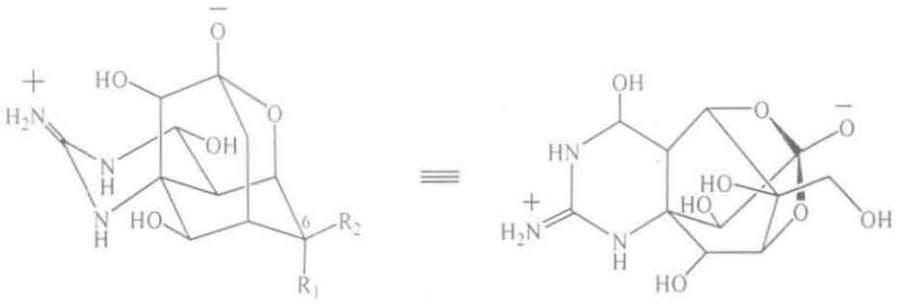
3

Tetrodotoxin (TTX, **4i**)

It is also a non-proteinaceous, highly toxic and guanidinium type compound. The name is derived from the family of puffer fish, *Tetraodontidae*. Both TTX and STX are the potent neurotoxins blocking $Na^+$ channel excitable membranes of the nerve, leading to paralysis. So they are extremely useful chemical tools for neurophysiology and neuropharmacology studies.[9a,b] It was previously isolated from the puffer fishes of Japan seas, as puffer fish poisoning was a social problem in Japan. Later, the toxigenic source of TTX was found in other animals like, Californian newt (*Taricha torosa*), crabs of *Xanthidae* family, Australian blue-ringed octopus (*Hapalochlaena maculosa*), Japanese ivory shell (*Babylonia japonica*), Indo-Pacific goby (*Ctenogobius criniger*), etc.[9c] Recently there is evidence that the causative organism for this toxin is the symbiotic bacteria including the family *Vibrionaceae*, *eg. Pseudomonas* spp. and *Photobacterium phosphoreum* present in these animals.[10]

The structural features of **4i** is provided below:

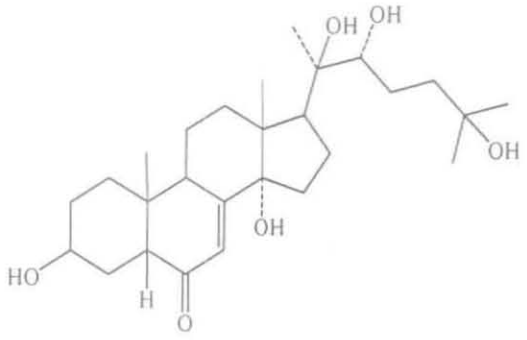
MF. $C_{11}H_{17}N_3O_8$; M.W. 319.27; Soluble in dilute acetic or sulfuric acid, less soluble in water, ethanol and diethylether and insoluble in organic solvents; CA name: 5,9:710a-Dimethano-10aH-[1,3]dioxocino[6,5-d]pyrimidine-4,7,10,11,12-pentol, octahydro-12-(hydroxymethyl)-2-imino-,



| | $R_1$ | $R_2$ |
|---|---|---|
| 4i, TTX | OH | $CH_2OH$ |
| 4ii, 6-*epi*-TTX | $CH_2OH$ | OH |
| 4iii, 11-deoxy TTX | OH | $CH_3$ |
| 4iv, 11-oxo TTX | OH | CHO |
| 4v, 11-nor TTX-6(*R*)-ol | H | OH |
| 4vi, 11-nor TTX-6(*S*)-ol | OH | H |

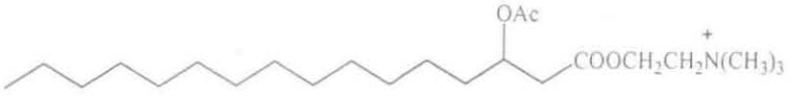
The structure of **4** was extensively studied.[11] Various TTX (**4ii** – **4vi**) analogues have also been characterized.[12]

Guanidine and amide bond containing compounds have been extracted from marine sources by aqueous phase containing little acid (~1%), as has been seen in the case of TTX from puffer fish and many compounds from invertebrates.[13] 2-Deoxycrustecdysone (**5**) was isolated from crayfish, *Jasus islandei* in trace quantity. **5** exhibits hormonal activity in the molting of crustaceans and also exhibits growth regulating activity in mariculture.[14]




5

3-Acetoxyhexadecanoyl choline, pahutoxin (**6**), was isolated from Hawaiian boxfish "Pahu", *Ostracion lentiginosus*[15] and it has surfactant and haemolytic properties.




6

Peptide toxins with leucine, phenylalanines as components were isolated from mucus producing glands of soapfish, *Rypticus saponaccus* and Pacific bass, *Grammistes, sexlineatus*. They are secreted as the defensive chemicals. Hashimoto *et al.*[16] fractionated three peptides, grammistins A, B and C from the toxin obtained from soapfish, *Pogonoperaca punctata*.

**Chemical Analysis of puffer fish, *Tetraodon* spp.**

100 kg of fresh dead *Tetraodon* spp. fishes (**Fig - 1**, ~500 nos) were procured during June 2002. They were dissected out to collect about 11 kg of internal organs[17] comprising liver, gonads and digestive tracts and frozen. They were later thawed and macerated in portions and extracted with 2.5 times volume of 0.1% aqueous acetic acid solutions in portions. The pH of slurry was adjusted to ~5.5 (pH paper) and digested over water bath. The fatty materials were skimmed off and the solution was filtered through a damp cloth to get rid of suspended impurities of intestine like clam shells. The aqueous extract was further acidified with acetic acid to arrest putrification and color change. The pH was maintained in slightly acidic side, until it was transported to the lab for further work. The light brown extract was tested by *tlc* (solvent system-EtOH:AcOH, 96:4). The scheme of extraction is shown in Fig-2

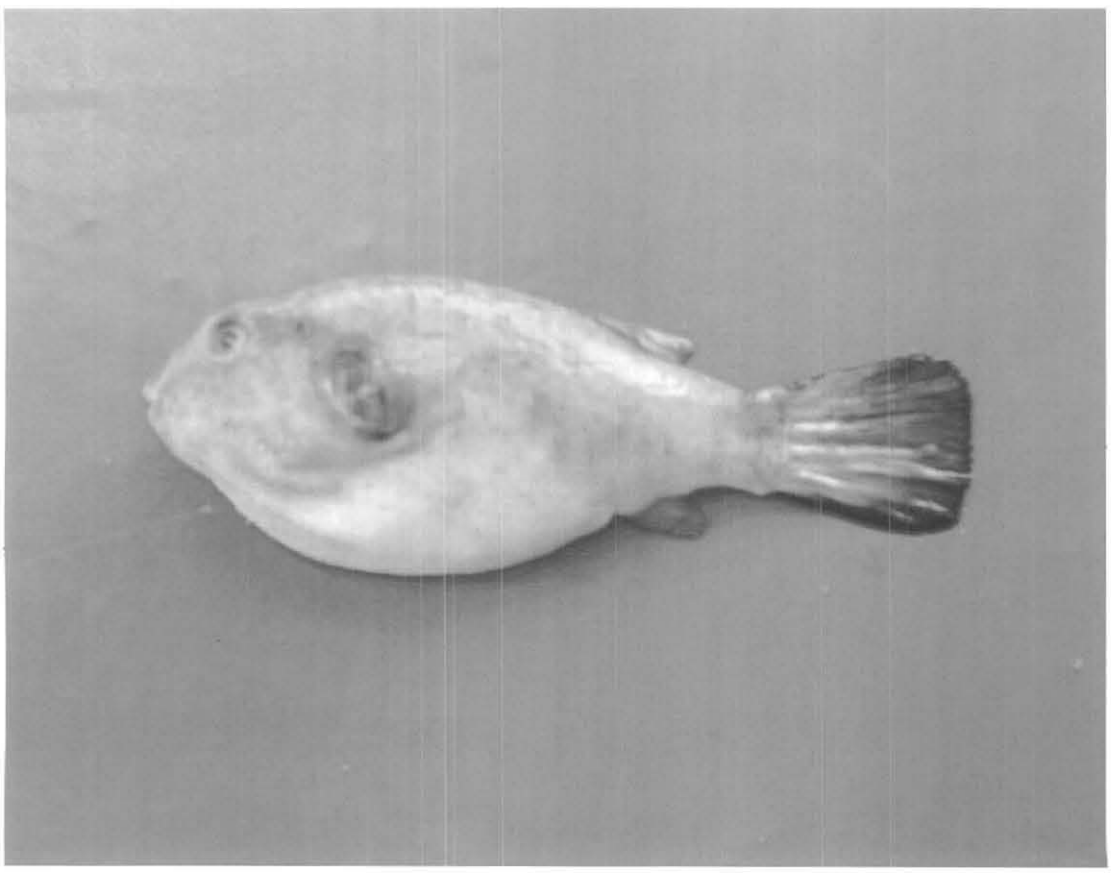
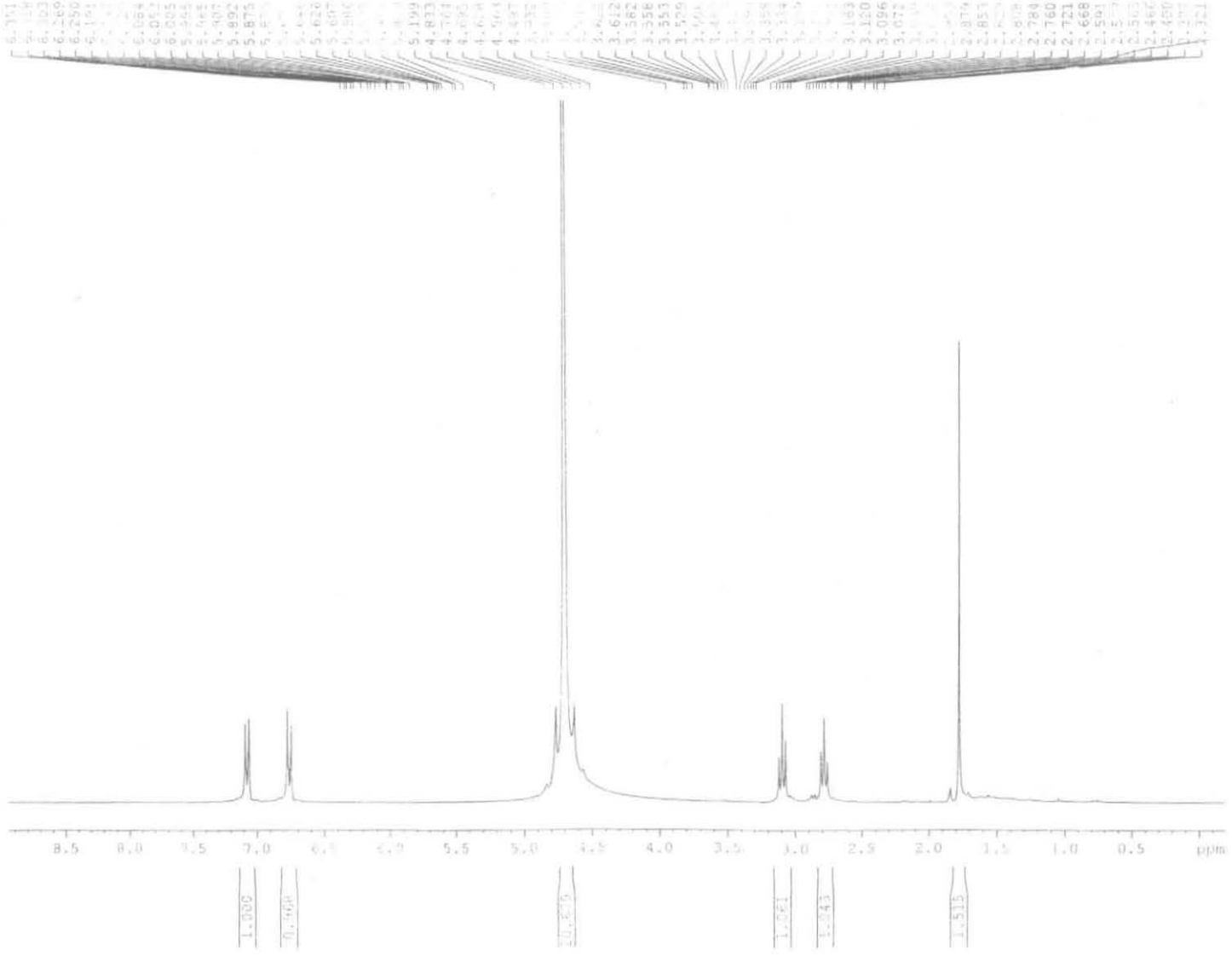


**Fig - 1**

The crude extract was concentrated at 40°C and then treated with ethanol. The insoluble grey mass appeared was filtered off. The resultant solution was then concentrated. To this concentrate, diethyl ether was added. Upon the gradual addition of ether a sticky brown mass got deposited. The diethyl ether layer was decanted from this mass and crystalline solid appeared on the walls of the tube. It was then filtered to give a crystalline hygroscopic solid, **8-1-1** (100 mg). The diethyl ether layer upon evaporation gave another liquid, **8-2-1** (175 mg). These two compounds have been analyzed for their structure by the combined use of spectroscopic techniques.

**Structural identification of 8-1-1**

As this compound is a hygroscopic solid, the melting point could not be determined. The $^{1}H$ NMR spectrum of **8-1-1** (**Fig - 2** and **3**) in $D_2O$ with TMS as standard is relatively simple. There is a pair of doublets at 7.0 ppm and 6.76 ppm with a coupling constant of 8.4 Hz. There is another pair of triplets at 3.10 ppm and 2.78 ppm each having a coupling constant of 7.2 Hz. Apart from these signals, a singlet appears at 1.78 ppm. The intensity ratio of these signals is 2:2:2:2:3. The coupling constant and H-H COSY spectrum (**Fig - 4**) clearly reveal the coupling connections. The $^{13}C$ NMR spectrum of **8-1-1** (**Fig - 5**) exhibits eight signals. From the DEPT experiment (**Fig - 6** and **7**), it can be seen that the signals at 41.0 and 32.2 ppm are due to methylene carbons; those at 135 and 116.1 ppm are due to methine carbons and that at 23.6 is due to methyl carbon. Obviously, the signals at 181.0, 154.9 and 128.7 are due to quaternary carbons. The C-H COSY experiment (**Fig - 8**) and HMBC experiment (**Fig - 9**) reveal one bond and multiple bond connectivity between the carbons and hydrogens. It is very clear that a *para* disubstituted phenyl unit, $X\text{-}C_6H_4\text{-}CH_2CH_2\text{-}Y$ and an acetyl group are there in the molecule. The chemical shift values of carbon and hydrogen and the presence of mass fragments at *m/e* more than 300 in the mass spectrum corner us to assign the structure of compound tentatively as shown in **Fig - 10**.

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PROCNO 1

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TD 32768
SOLVENT D2O
NS 25
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SWH 6172.839 Hz
FIRES 0.188380 Hz
AQ 2.6542580 sec
RG 128
DW 81.000 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec

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PL1 0.00 dB
SFO1 300.1318534 MHz

F2 - Processing parameters
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WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Fig – 2 $^{1}$H NMR spectrum of 8-1-1

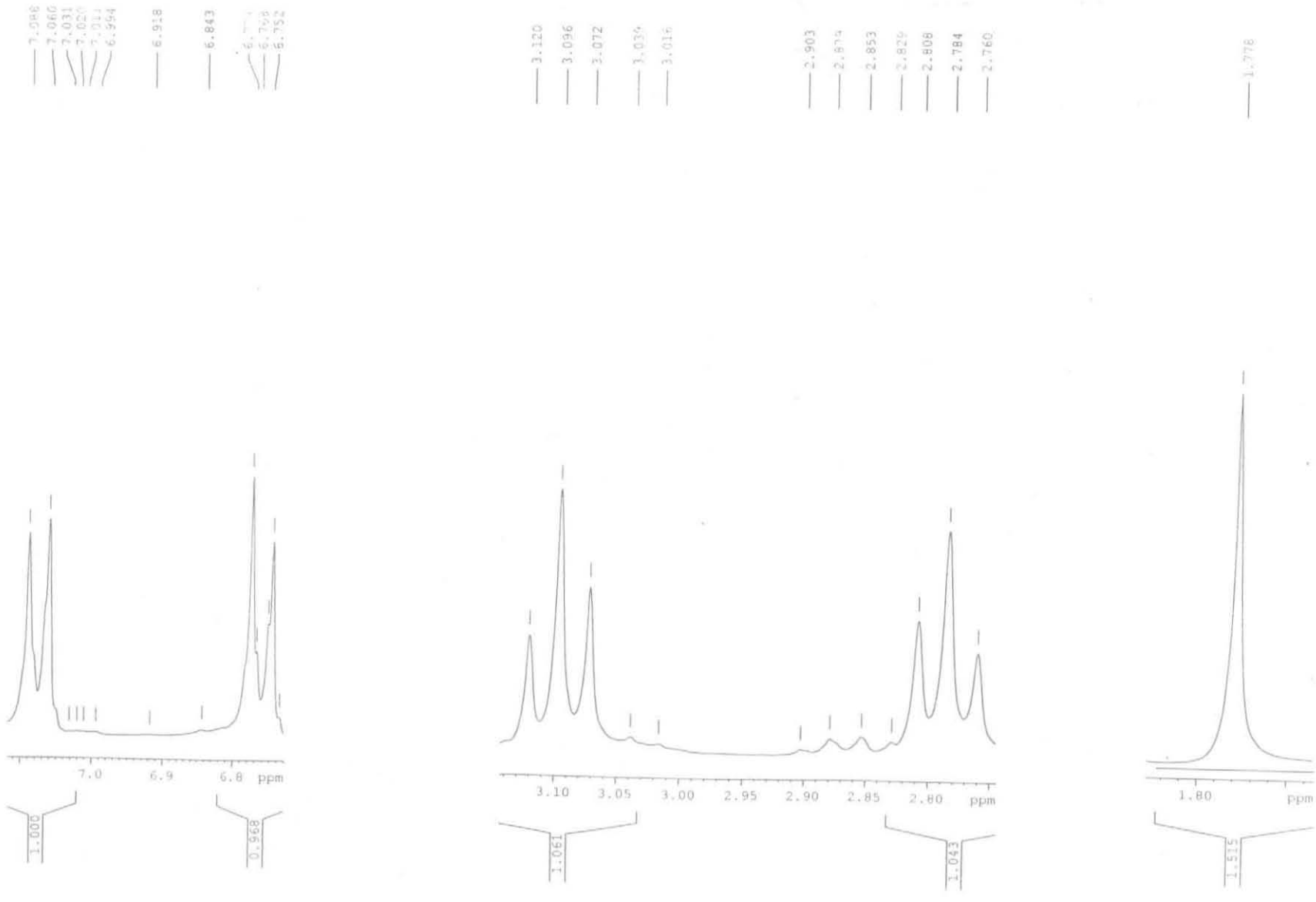



Fig - 3 $^{1}$H NMR spectrum of 8-1-1 (Expanded)

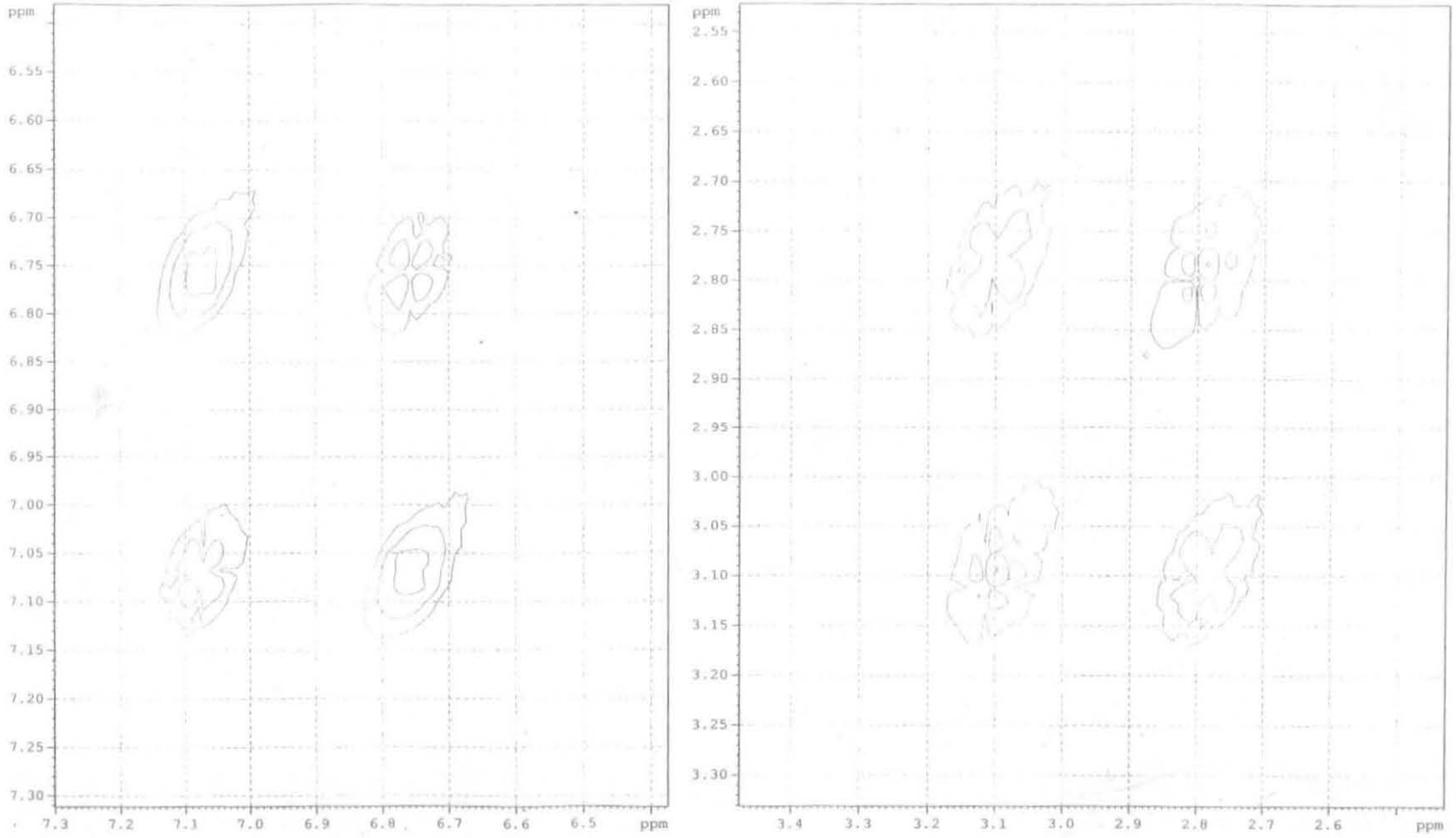
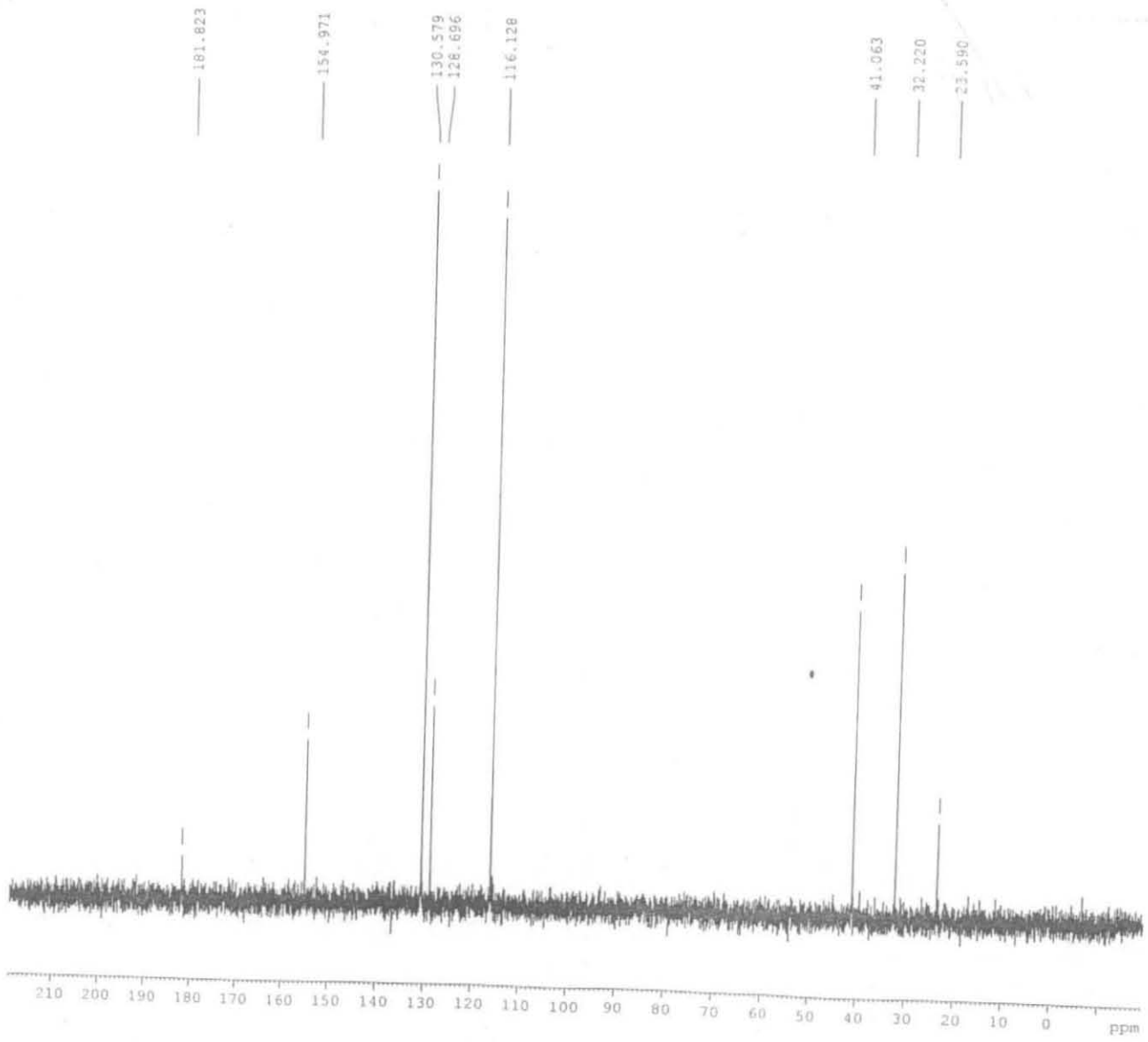



**Fig – 4 H-H COSY spectrum of 8-1-1**

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PROCNO 1

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SOLVENT D2O
NS 1024
DS 4
SWH 17985.611 Hz
FIDRES 0.274439 Hz
AQ 1.8219508 sec
RG 16384
DW 27.800 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
d11 0.03000000 sec
d12 0.00002000 sec

======== CHANNEL f1 ========
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P1 6.80 usec
PL1 -4.00 dB
SFO1 75.4752653 MHz

======== CHANNEL f2 ========
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PCPD2 80.00 usec
PL2 0.00 dB
PL12 17.68 dB
PL13 20.00 dB
SFO2 300.1312005 MHz

F2 - Processing parameters
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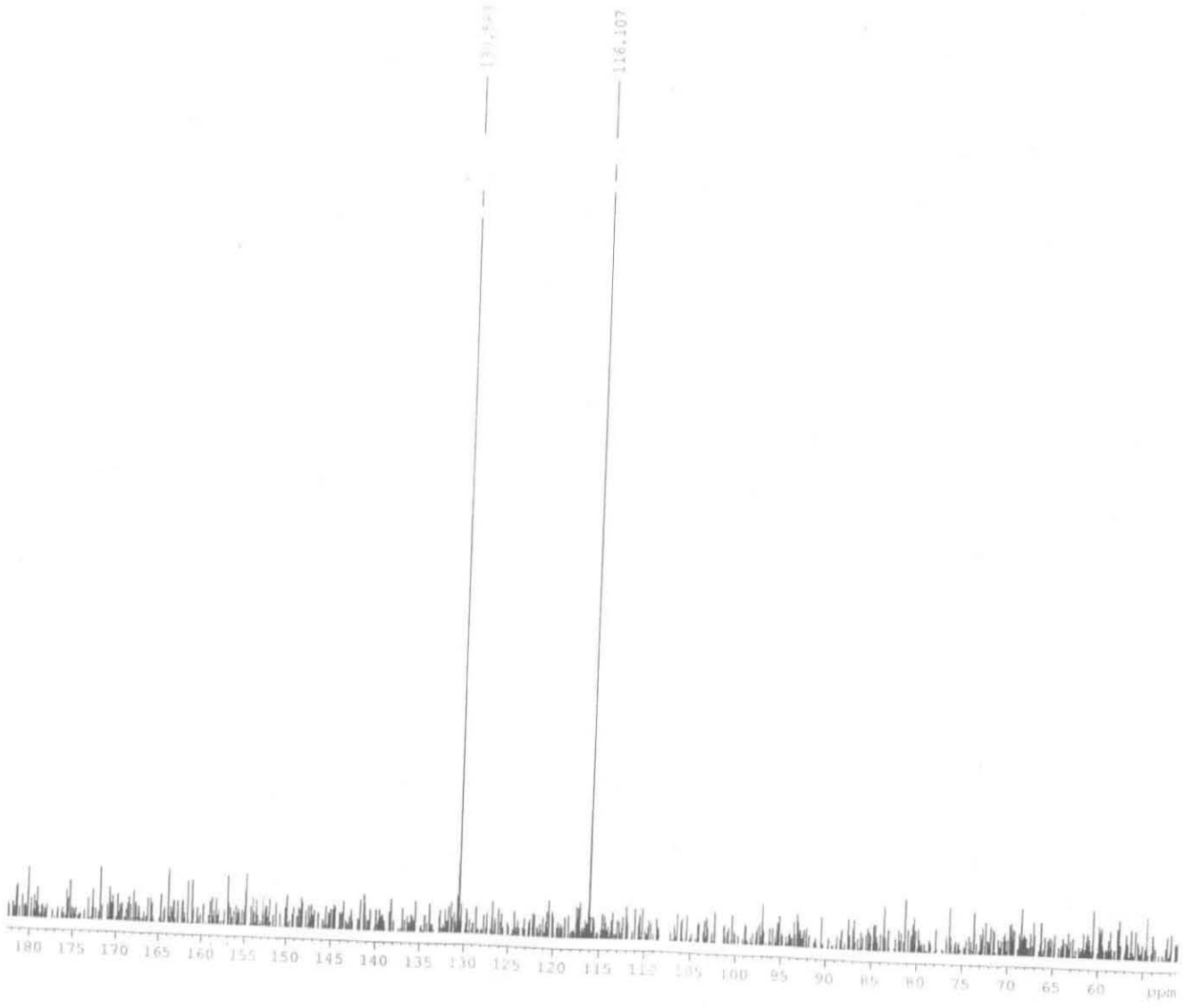
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

Fig - 5 $^{13}C$ NMR spectrum of 8-1-1

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PROCNO 1

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PULPROG dept90
TD 65536
SOLVENT D2O
NS 56
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SWH 17985.611 Hz
FIDRES 0.274439 Hz
AQ 1.8219508 sec
RG 16384
DW 27.800 usec
DE 6.00 usec
TE 300.0 K
CNST2 145.0000000
D1 2.00000000 sec
d2 0.00344828 sec
d12 0.00002000 sec
DELTA 0.00000866 sec

CHANNEL f1
NUC1 13C
P1 6.80 usec
p2 13.60 usec
PL1 -4.00 dB
SFO1 75.4752653 MHz

CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
P3 10.45 usec
p4 20.90 usec
PCPD2 80.00 usec
PL2 0.00 dB
PL12 17.68 dB
SFO2 300.1312005 MHz

F2 - Processing parameters
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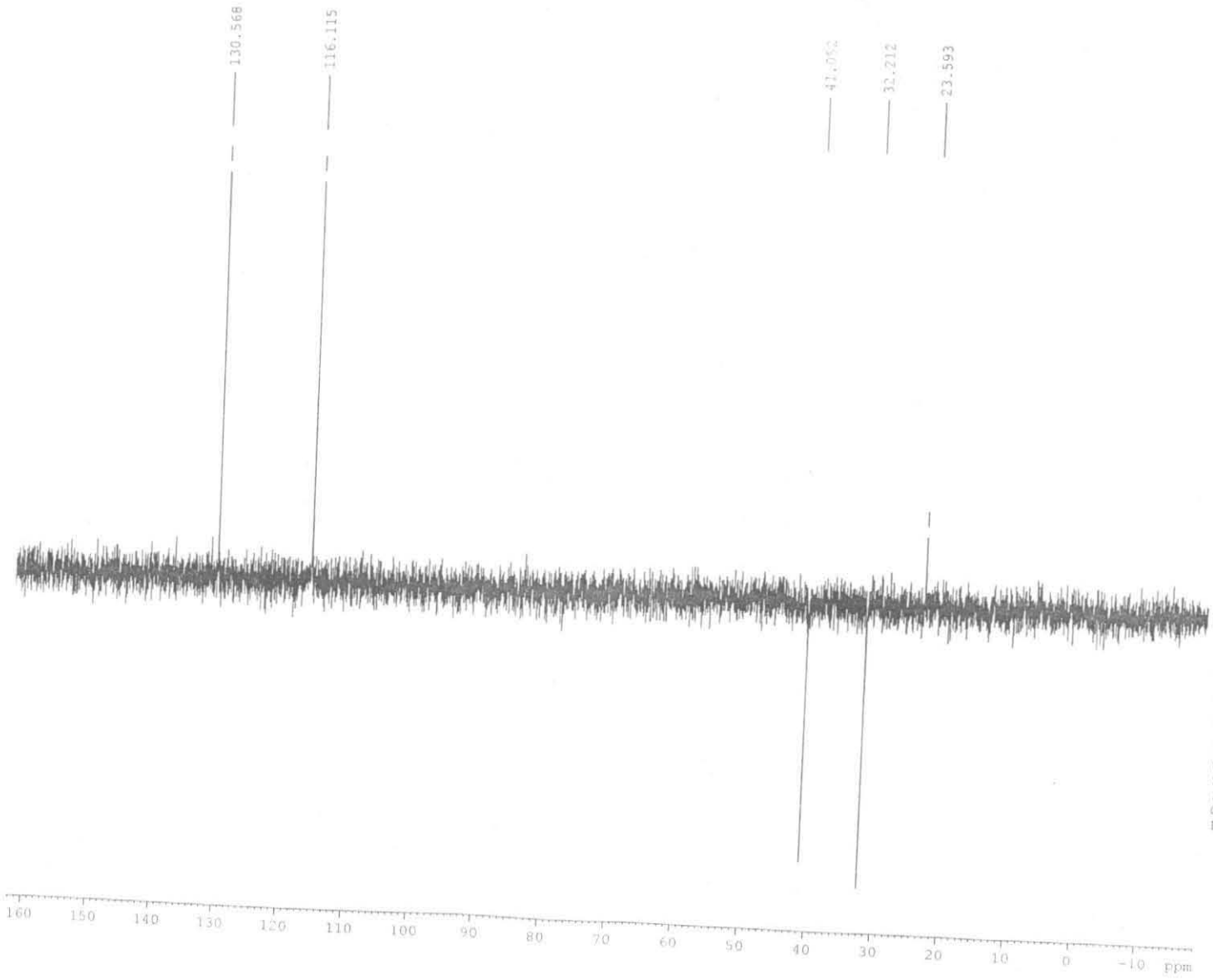
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

**Fig - 6 DEPT 90 experiment of S-1-1**

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PROCNO 1

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TD 65536
SOLVENT D2O
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DE 6.00 usec
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p2 13.60 usec
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SFO1 75.4752653 MHz

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NUC2 1H
P3 10.45 usec
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FL2 0.00 dB
PL12 17.68 dB
SFO2 300.1312005 MHz

F2 - Processing parameters
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**Fig – 7 DEPT 135 experiment of 8-1-1**

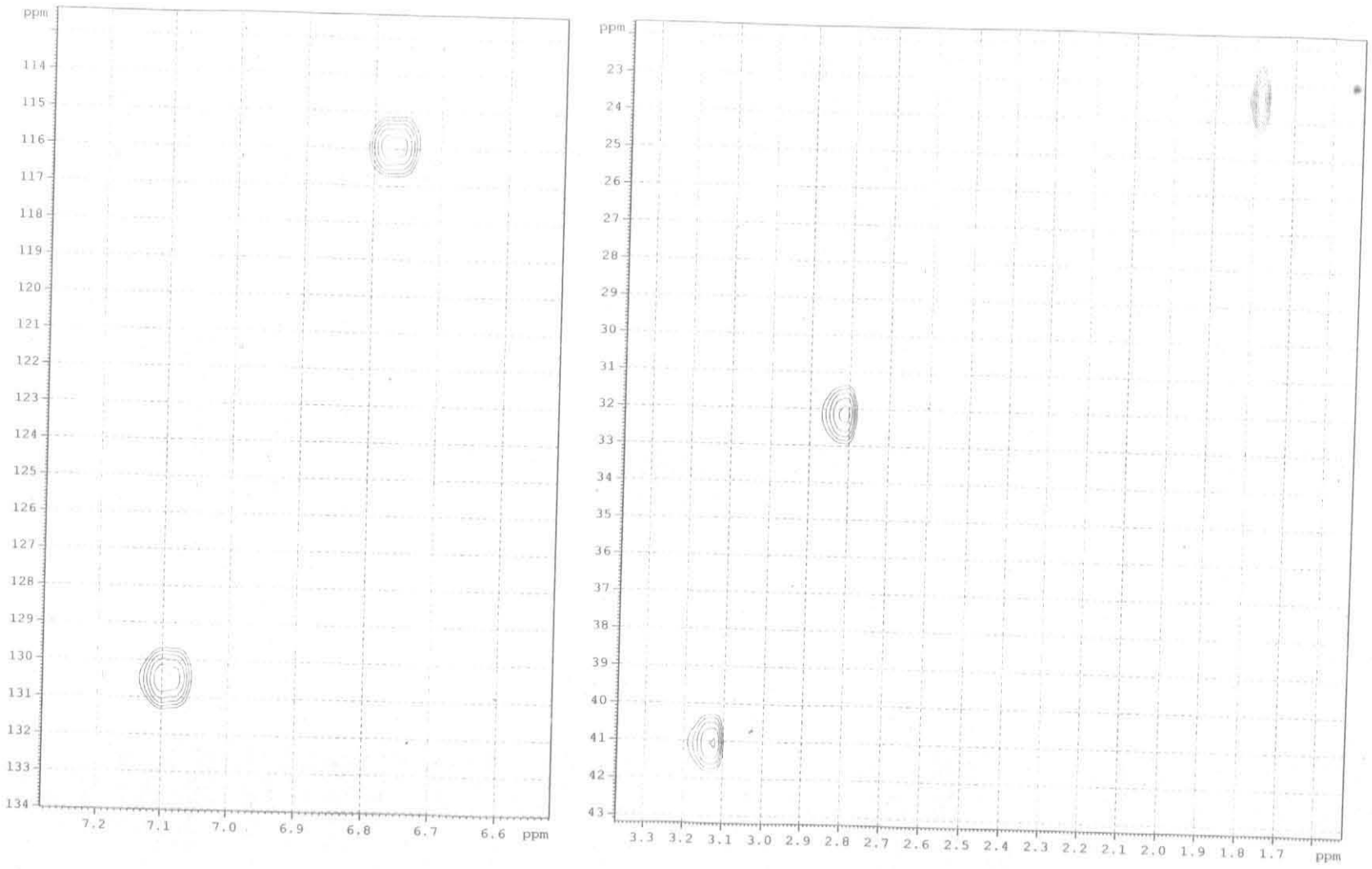



**Fig – 8 C-H COSY experiment of 8-1-1**

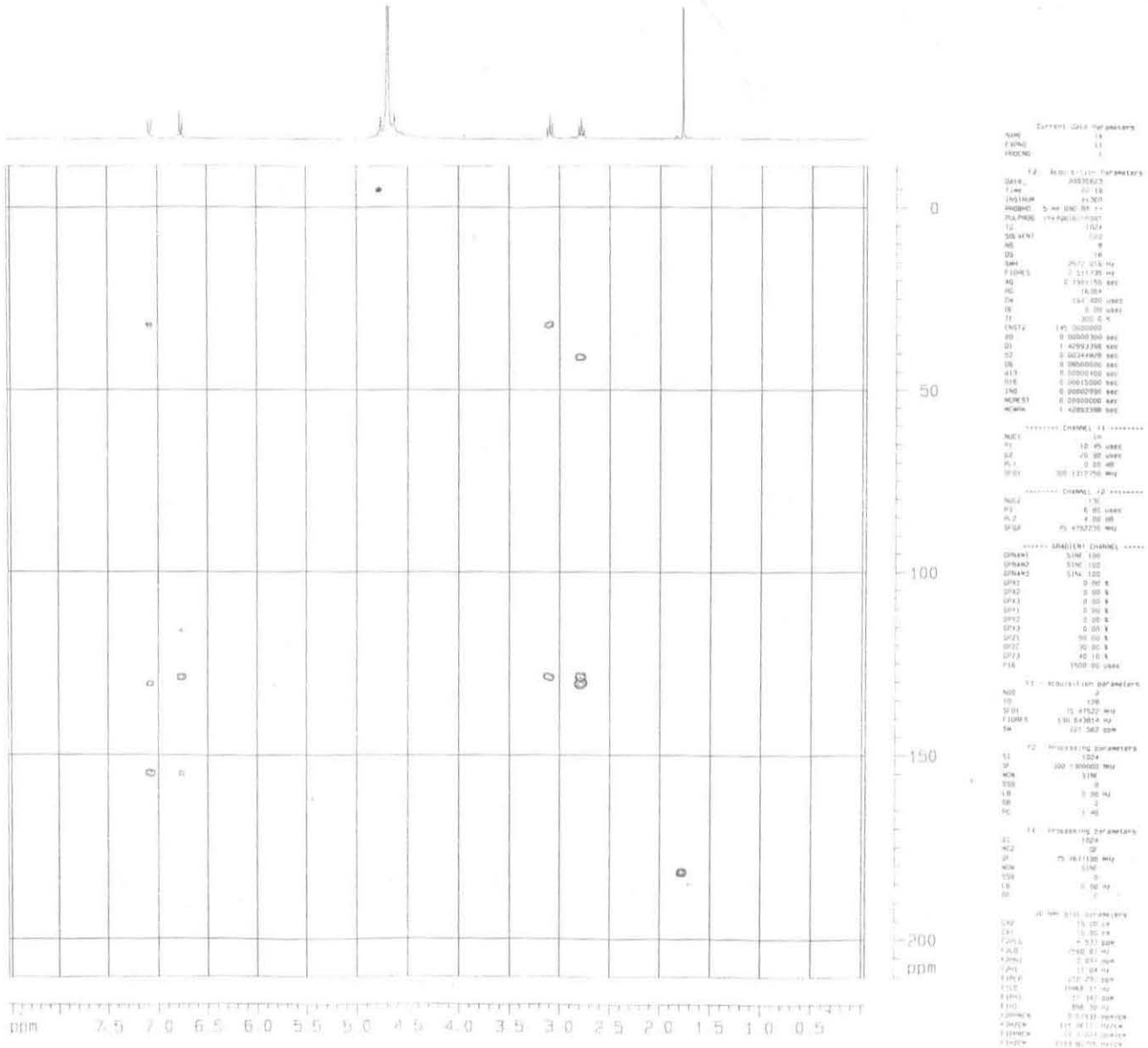



**Fig – 9 HMBC experiment of 8-1-1**

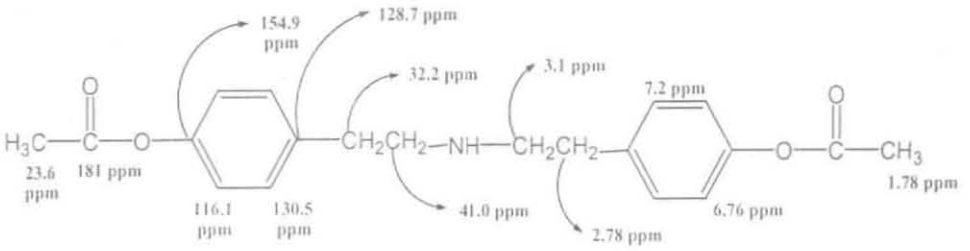



Fig - 10

**Structural identification of compound 8-2-1**

The mass spectrum of **8-2-1** (**Fig - 11**) perfectly matches with that of piperidin-2-one. The $^{1}H$ (**Fig - 12, 13**) and $^{13}C$ NMR spectrum (**Fig - 14**) H-H COSY, DEPT (**Fig - 15, 16**) all support this assignment (**Fig - 17**).

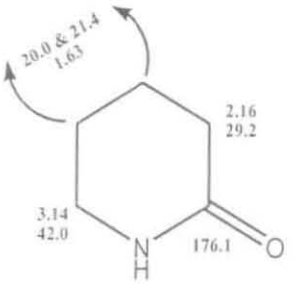
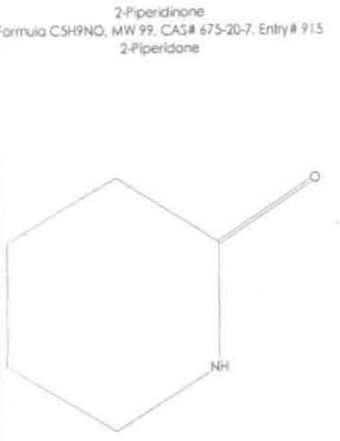
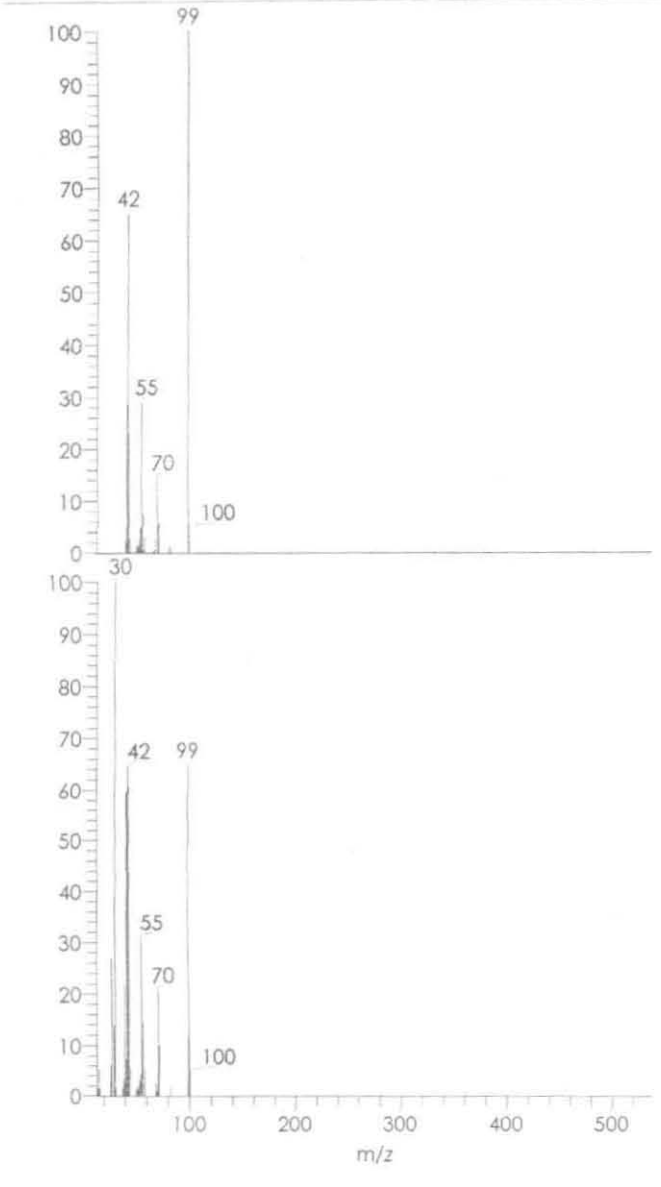



**Fig - 17**

Thus two simple organic compounds have been isolated and tentatively characterized from the aqueous acidic extract of the puffer fish, *Tetraodon* spp.

| Hit | SI | RSI | Name | Library Name |
|---|---|---|---|---|
| 1 | 866 | 873 | 2-Piperidinone | |
| 2 | 858 | 860 | 2-Piperidinone | |
| 3 | 756 | 762 | 1-Pyrrolidineca | |
| 4 | 733 | 734 | 2-Pyrrolidinone | |
| 5 | 729 | 738 | δ Nonalactone | |
| 6 | 725 | 749 | 3,5-Diamino-1, | |
| 7 | 717 | 737 | Glycocyanidin | |
| 8 | 709 | 719 | Hexanoic acid | |
| 9 | 707 | 708 | 2H-Pyran-2-on | |
| 10 | 703 | 726 | 1-Pyrrolidineca | |
| 11 | 703 | 712 | Hexanoic acid | |
| 12 | 702 | 703 | 2H-Pyran-2-on | |
| 13 | 695 | 695 | 2H-Pyran-2-on | |
| 14 | 690 | 695 | 2H-Pyran-2-on | |
| 15 | 686 | 746 | 2,5-Pyrrolidinec | |
| 16 | 686 | 706 | 2,5-Pyrrolidinec | |
| 17 | 680 | 688 | 1H-Azepine, he | |
| 18 | 678 | 689 | 2,5-Pyrrolidinec | |

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SI 866, RSI 873, MAINLIB, Entry# 915, CAS# 675-20-7, 2-Piperidinone

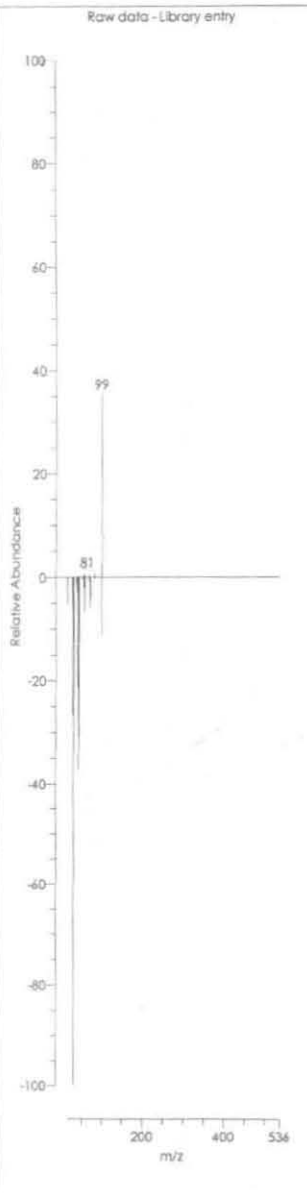
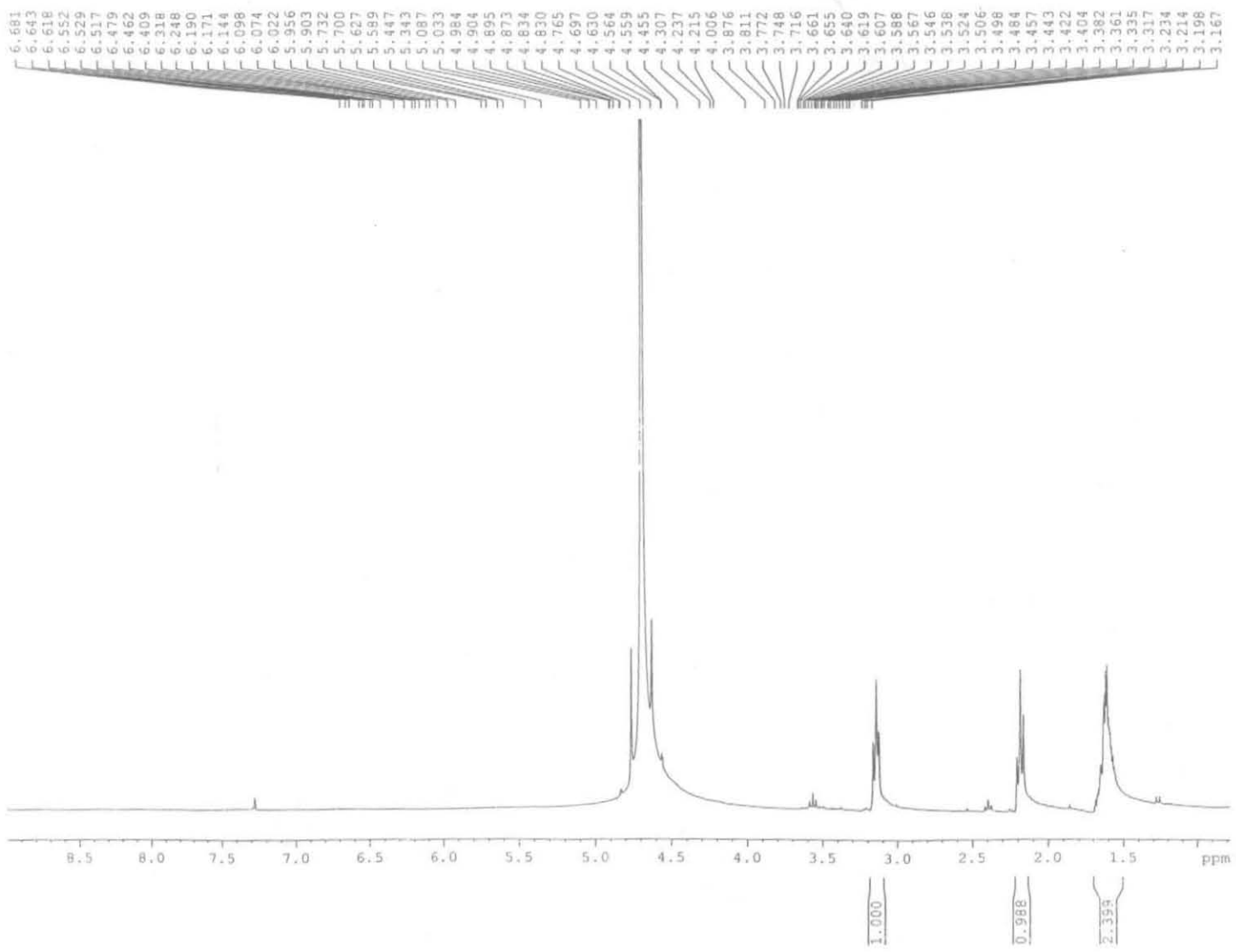



Fig – 11 Mass spectrum of 8-2-1

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FIDRES           0.188380 Hz
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RG                      64
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D1             2.00000000 sec

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Fig – 12 $^{1}$H NMR spectrum of 8-2-1

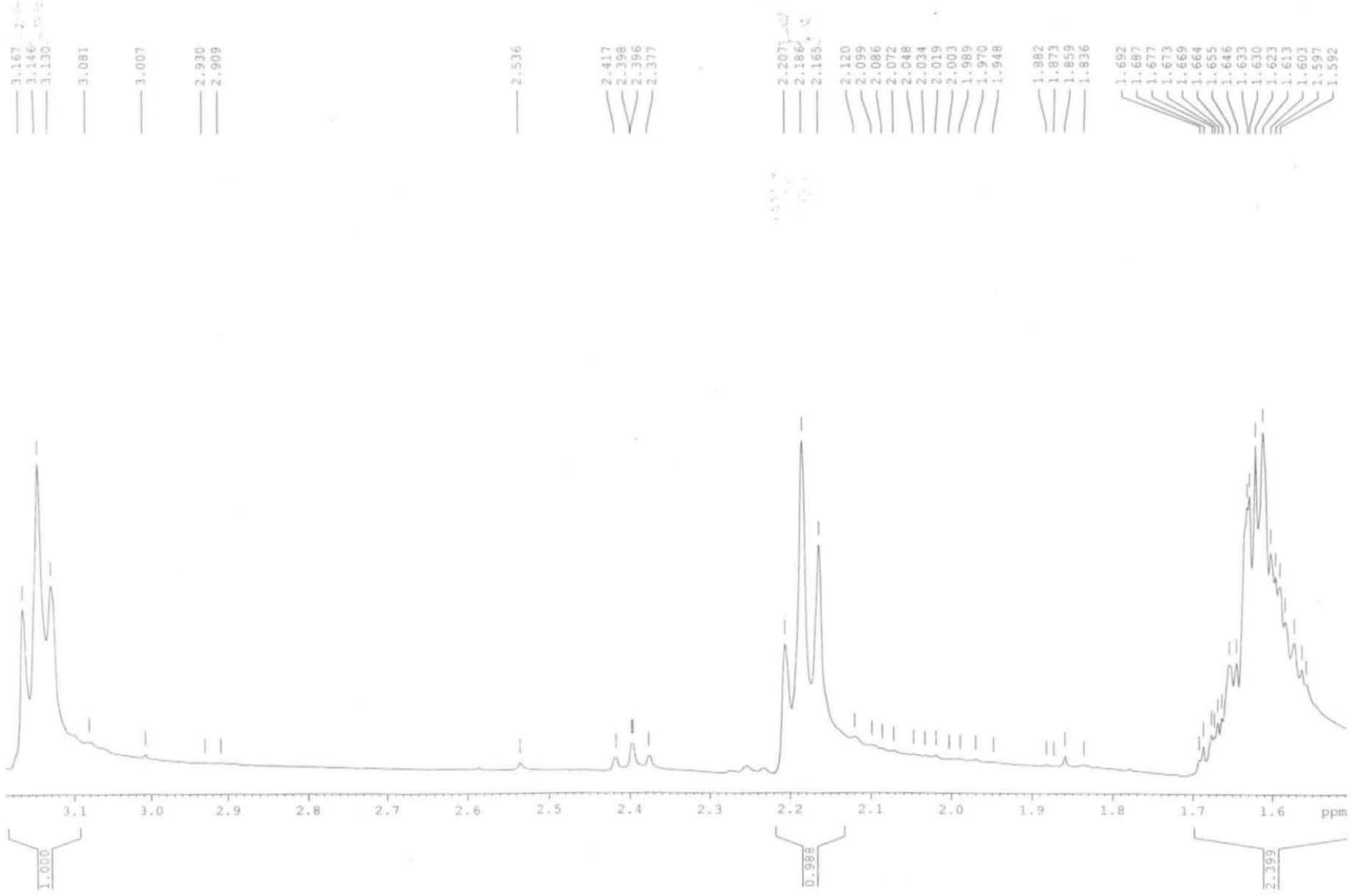
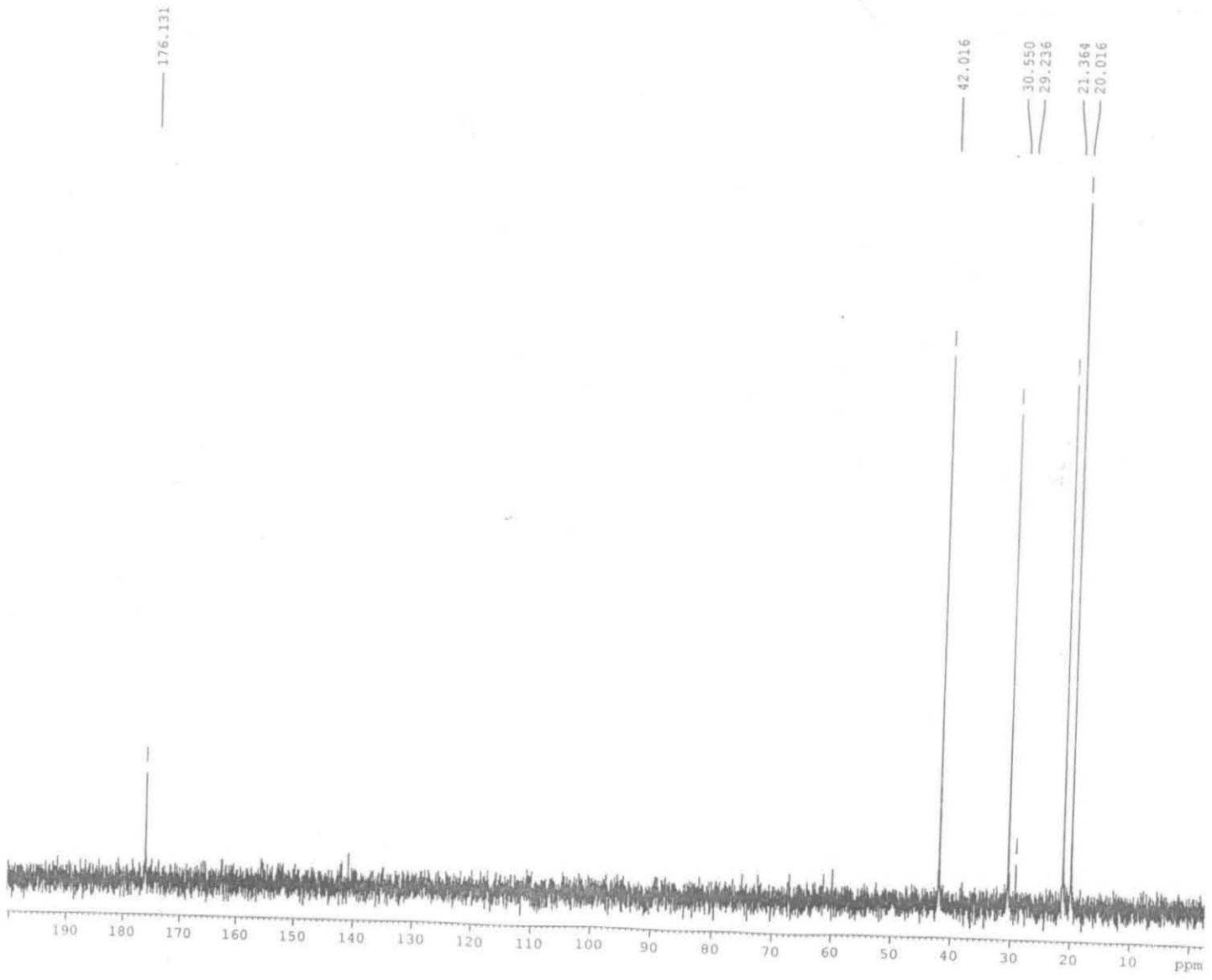



Fig – 13 $^{1}$H NMR spectrum of 8-2-1 (Expanded)

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FIDRES 0.274439 Hz
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RG 6502
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DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
d11 0.03000000 sec
d12 0.00002000 sec

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P1 6.80 usec
PL1 -4.00 dB
SFO1 75.4752653 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 0.00 dB
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PL13 20.00 dB
SFO2 300.1312005 MHz

F2 - Processing parameters
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Fig – 14 $^{13}$C NMR spectrum of 8-2-1

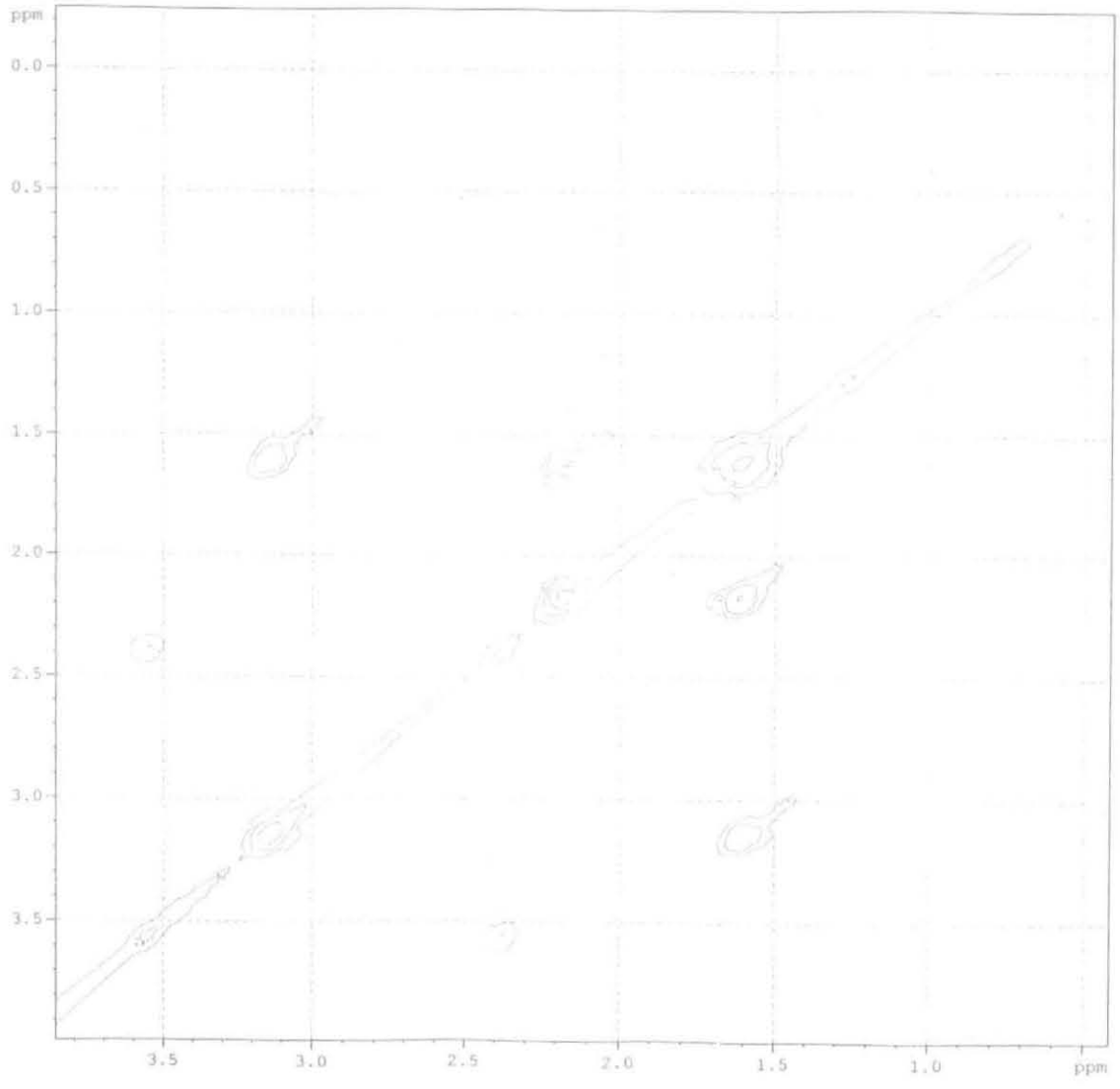


Fig – 15 H-H COSY experiment of 8-2-1

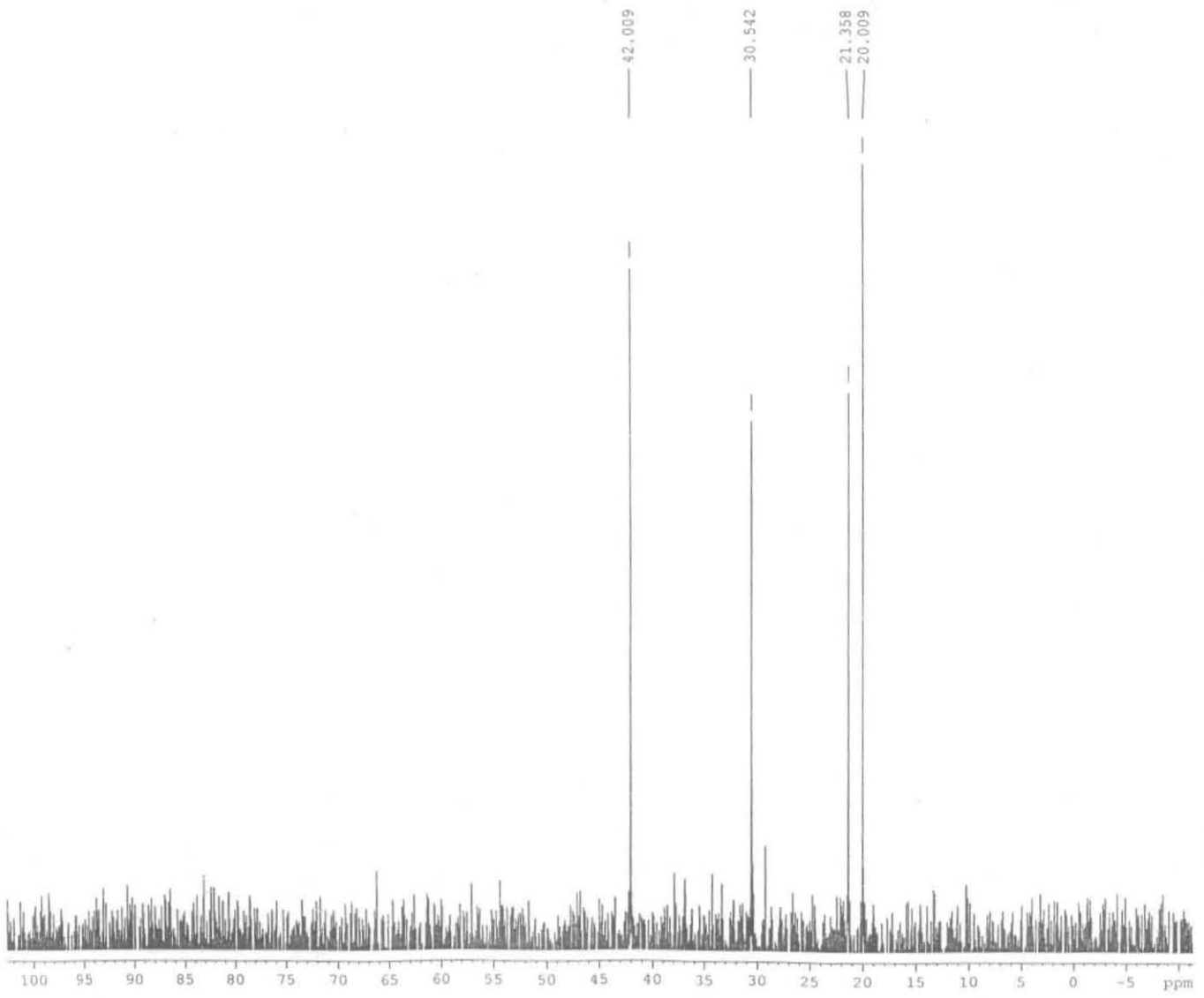


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**Fig – 16 DEPT 135 experiment of 8-2-1**

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11. (a) T Goto, Y Kishi, S Takahashi & Y Hirata, *Tetrahedron*, 1965, **21**, 2059; (b) RB Woodward, *Pure Appl. Chem.*, 1964, **9**, 49.
12. (a) T Yasumoto, M Yotau, M Murata & H Naoki, *J. Am. Chem. Soc.*, 1989, **110**, 2344; (b) A Endo, SS Khora, M Murata, H Naoki & T Yasumoto, *Tetrahedron Lett.*, 1988, **29**, 4127; (c) SS Khora & T Yasumoto, *Tetrahedron Lett.*, 1989, **30**, 4393; (d) M Yotsu, T Yasumoto, YH Kim, H Naoki & CY Kao, *Tetrahedron Lett.*, 1990, **31**, 3187; (e) M Yotsu, Y Hayashi, SS Khora, S Sato & T Yasumoto, *Biosci. Biotech. Biochem.*, 1992, **56**, 370.
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Chapter 9

# Synthesis of thiazolidine derivatives and their evaluation for antifungal activity

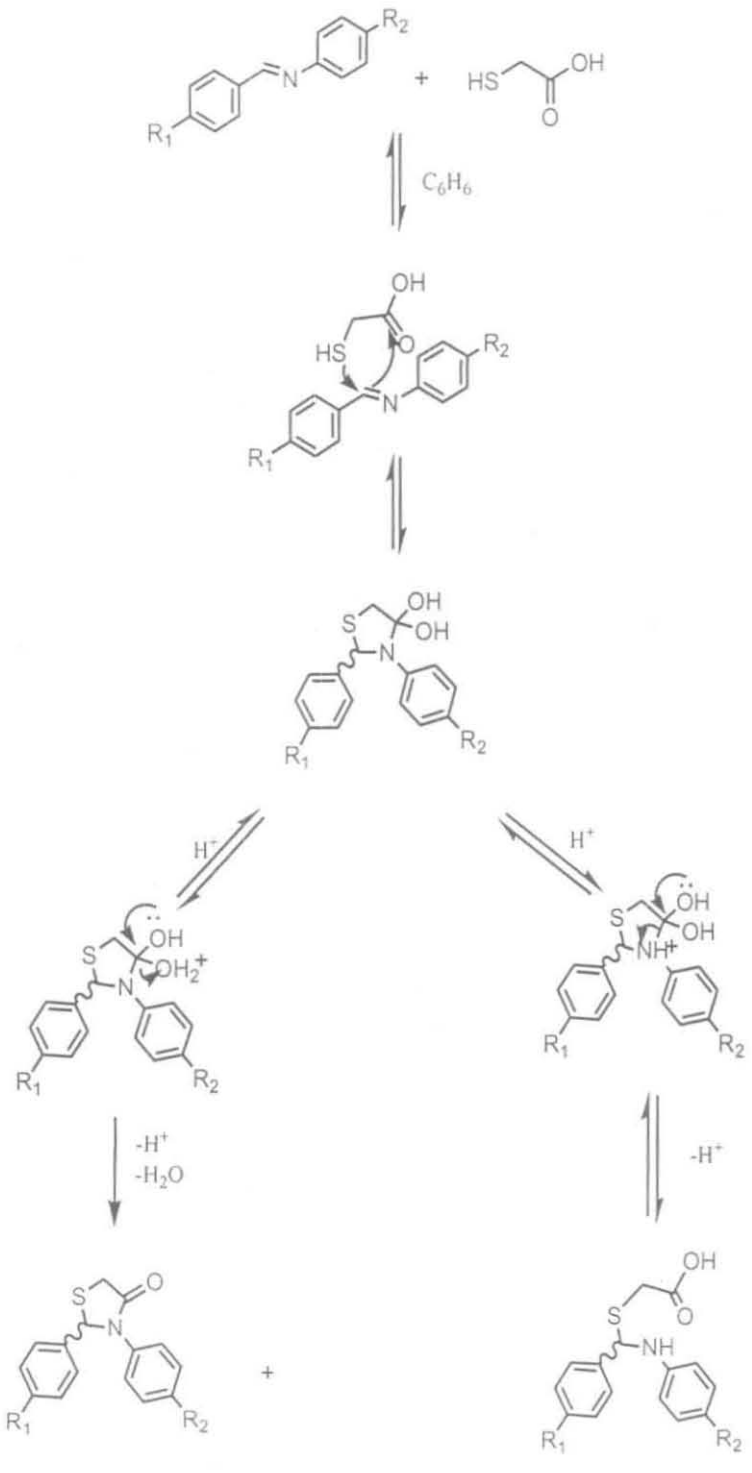
Several five membered heterocyclic compounds have been found to be present as components in many marine natural products. Five membered nitrogen heterocycles[1,2], oxygen heterocycles[3] and cyclic polysulphides[4] have been isolated from different marine sources. A thiazole containing compound mycothiazole[5] has been isolated from the dichloromethane soluble fraction of the methanol extract of *S. mycofijiensis*. As major part of this thesis deals with isolation and identification of organic compounds present in marine natural products, it is felt worthy to synthesize some organic compounds having nuclei found in marine products. Hence, it has been planned to synthesize compounds having five membered systems with more than one heteroatom possessing potential biological activity. This chapter of the thesis describes the synthesis, characterization and antifungal characteristics of a new set of thiazolidinone system.

It is appropriate to review the importance and the popular method of preparing thiazolidinone rings and hence a brief survey on the recent reports is presented in the following pages.

The reaction of thioglycollic acid with imines normally leads to 1,3-thiazolidin-4-one. The reaction of phosphorylated thiourea with methyl chloroacetate also gives 1,3-thiazolidin-4-one. Total synthesis of a series of thiazolinone and thiazolidinone analogues of the antibacterial oxazolinone antibiotic indolmycin is recently described. The synthetic route involves the nucleophilic displacement of mesyloxy and chloro groups from methyl 2-substituted-3-(indol-3-yl)propionates and butyrates with N-substituted thioureas.[6] 2,3-diaryl-1,3-thiazolidin-4-ones have been prepared from azomethines and $\alpha$-mercaptoacetic acid and tested for anti-inflammatory and antinociceptive activities. A good level of activity against carrageenan induced edema in rat hind paw has been observed.[7] Some 2-substituted phenyl-3-(N,N-dimethylaminopropyl)-1,3-thiazolidin-4-ones have been prepared and tested for antihistamine activity. The results showed an interesting degree of activity.[8] $^{1}H$ and $^{13}C$ NMR spectral analysis of several 3-benzyl-2-phenyl-1,3-thiazolidin-4-one have been carried out and good Hammett correlation has been observed between the chemical shifts of geminal benzylic protons.[9]

The reaction between N-(anthracen-9-yl)-N'-ethylthiourea and methylbromoacetate yielded differently substituted 1,3-thiazolidin-4-ones. The structures of the products were elucidated by the NMR and X-ray crystallographic analysis.[10] Several 2,3-diaryl-1,3-thiazolidine-4-ones have been synthesized from appropriate Schiff's bases and α-mercaptoacetic acid and screened for antimicrobial activity against *S. aureus, S. beta-haemoliticus, B. subtilis, M. paratuberculosis 607, S. typhi, Kl. pneumoniae, E. coli Bb, Ps. aeruginosa, C. albicans, A. niger, S. cerevisiae* by a disk-diffusion assay (Kirby-Bauer modified). The results obtained in this investigation showed that the prepared compounds exhibit varying degrees of antimicrobial activity.[11] The mass spectral study of some 2,3-diaryl-1,3-thiazolidin-4-one has been carried out and the major fragment ions were identified. Here again, good correlations were observed between the relative abundance of the fragments and Hammett σ constants of substituents in the aromatic rings.[12] Very recently, anti-HIV characteristics have been noticed in some 2,3-diaryl-1,3-thiazolidin-4-ones when they were subjected to QSAR study.

In the present study, some new 1,3-thiazolidin-4-ones have been prepared and the synthesis has been carried out under microwave irradiation. It is interesting to quote a recent study on the microwave assisted synthesis of thiazolidin-4-one, in which the effect of the solvents in governing the mechanism of the reactions has been investigated.[13] It has been shown that the microwave assisted reaction between Schiff's base and mercaptoacetic acid is more efficient and faster to produce thiazolidin-4-ones than the conventional method. It has also been revealed that in benzene, the mechanism of the reaction is shown as in **Scheme - 1** while in DMF the mechanism is as described in **Scheme - 2**.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OH
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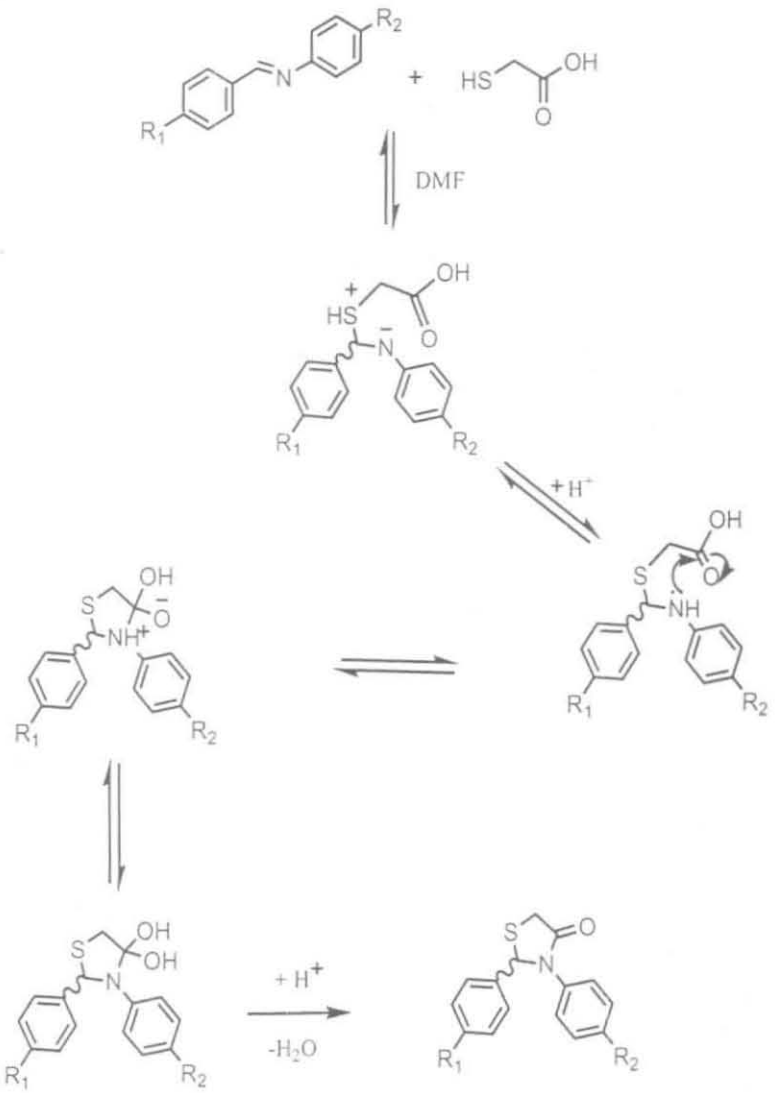
R1
R2

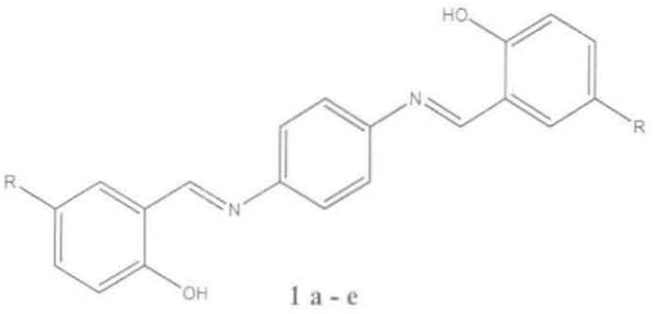
**Scheme - 1**




**Scheme - 2**

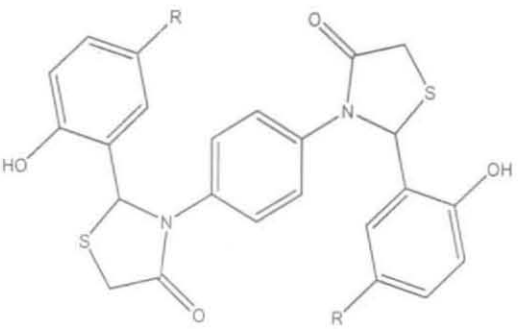
Having realized the biological importance of thiazolidin-4-one, in the present investigation, it has been planned to prepare such compounds by microwave irradiation from diimines using thioglycollic acid. In the synthetic group of this laboratory, recently several diimines have been prepared which have been subjected to a novel sodium borohydride reduction to get secondary amines.[14] Some of these diimines (**1a – 1e**) have been chosen as the starting material for the present work. Interestingly, there are two imine functionalities and hence there is a possibility of constructing two thiazolidin-4-one rings simultaneously. Presence of two thiazolidine rings may have added biological activity and hence the resultant products are expected to be doubly active.

With this in mind, the diimines **1a – 1e** have been treated with two equivalents of thioglycollic acid and the resultant paste was subjected to microwave irradiation for 10 minutes at 60 % power. Quick decolourisation occurred and very good yield of the dimeric thiazolidine **2** product has been obtained (**Scheme 3**). It is interesting to note that there is no 1:1 addition product. The yield and the melting point of the products **2a - 2e** are summarized in **Table - 1.**




**1 a - e**

$HSCH_2COOH$




**2a - e**

a) $R = CMe_2Ph$
b) $R = CMe_3$
c) $R = CMe_2Et$
d) $R = Et$
e) $R = CHMe_2$

**Scheme - 3**

**Table 1**: Yield and melting point of **2**

| Compound | Yield, % | *m.p.* °C |
|---|---|---|
| **2a** | 73 | 160-61 |
| **2b** | 72 | 149-50 |
| **2c** | 80 | 147-48 |
| **2d** | 75 | 142-43 |
| **2e** | 74 | 140-41 |

Attempted 1:1 reaction under microwave irradiation by mixing equimolar amount of diimine **1** and thioglycollic acid gave a mixture of several products, one of which has been identified as compound **2**.

[The typical experimental procedure for the above reaction is as follows: An intimate paste of thioglycollic acid (92 mg, 4 mmole) and the diimine, 2-({[4-({(E)-[2-hydroxy-5-(1-methyl-1-phenylethyl)phenyl]methylidene}amino)phenyl]imino}-methyl) -4-(1-methyl-1-phenylethyl)benzenol, **1a,** (1.1 g, 2 mmole), has been made and the resultant paste taken in a petri dish was irradiated in a domestic microwave oven for 10 minutes under 60 % power. The intense yellow color of the mixture vanished and the mass became colourless. The reaction mixture was then washed with water and little chloroform. It was then recrystallized from ethanol to get pure thiazolidinone, 2-[2-hydroxy-5-(1-methyl-1-phenylethyl)phenyl]-3-(4-{2-[2-hydroxy-5-(1-methyl-1-phenylethyl)phenyl]-4-oxo-1,3-thiazolan-3-yl}phenyl)-1,3-thiazolan-4-one, **2a**, (1.02 g, 73% yield).

In the same way, the other dimines **1b, 1c, 1d** and **1e** were treated with thioglycollic acid under microwave condition to get the respective thiazolidinones **2b-2e**. The yield, melting point and the spectral features are presented in **Tables-1** and **2**.

The starting material for the reaction **1a-1e** were all prepared from *p*-phenylenediamine and different 2-hydroxy-5-substituted benzaldehydes as described in a previous report.[14] 2-Hydroxy-5-substituted benzaldehydes, in turn were prepared by Reimer Tiemann reaction of the respective 4-susbstituted phenols.[15]]

The compounds **2** have been characterized by NMR spectroscopy. The $^{1}H$ and $^{13}C$ NMR spectral data is summarized in **Table - 2**.

**Table - 2**: NMR data of **2**

| Compd. | $^{1}H$ NMR data | $^{13}C$ NMR data |
|---|---|---|
| **2a** | 1.63, 12H, s; 3.38, 2H, d (15 Hz); 3.72, 2H, d (15 Hz); 5.67, 2H, s; 6.60-7.40, 20H, m | 30.7, 34.2, 42.1, 47.2, 115.4, 118.3, 123.9, 125.4, 126.0, 126.6, 127.4, 127.8, 135.8, 141.3, 150.7, 151.9, 172.0 |
| **2b** | 1.38, 18 H, s; 3.26, 2H, d (15 Hz); 3.38, 2H, d (15 Hz); 5.62, 2H, s; 6.60-7.50, 10H, m | 31.2, 33.8, 34.4, 47.4, 117.4, 115.8, 124.5, 125.6, 126.4 136.6, 139.2, 152.0 and 172.0 |
| **2c** | 0.65, 6H, t (7.2 Hz); 1.23, 12 H, s; 1.58, 4H, q (7.2 Hz); 3.22, 2H, d (15.3 Hz); 3.44, 2H, d (15.3 Hz); 5.69, 2H, s; 6.66, 4H, s; 6.78, 2H, d (8.4 Hz); 7.04, 2H, dd (8.4 and 2.1 Hz); 7.38, 2H, d (2.1Hz) | 9.1, 28.5, 34.3, 36.7, 37.1, 47.2, 115.3, 118.1, 124.0,125.3, 126.3, 136.1, 139.9, 151.6, 171.9 |
| **2d** | 1.20, 4H, q (7.2 Hz); 2.54, 6H, t (7.2 Hz), 3.24, 2H, d (15 Hz); 3.32, 2H, d (15 Hz); 5.65, 2H, s; 6.60-7.50, 10H, m | 15.98, 27.83, 34.20, 46.80, 115.5, 117.9, 124.9, 127.0, 128.2, 134.6, 144.4, 151.5, 170.0 |
| **2e** | 1.20, 12H, d (6.6 Hz); 2.82, 2H, septet (6.6 Hz); 3.27, 2H, d (15 Hz); 3.47, ppm,2H, d (15 Hz); 5.68, 2H, s; 6.58, 4H, s; 7.31, 2H, s 6.96, 2H, d (8.4 Hz); 6.77, 2H, d, (J = 8.4 Hz). | 24.1, 33.1, 34.4, 47.3, 117.4, 115.8, 124.6, 125.6, 126.6 136.6, 139.5, 152.0 and 172.0 |

The $^{1}H$ NMR spectrum of **2e** (**Fig - 1** and **2**) exhibits a doublet at 1.20 ppm with a coupling constant of 6.6 Hz and a one hydrogen septet at 2.82 (J = 6.6 Hz). An AB quartet has been noticed at 3.27 and 3.47 ppm, each having a coupling constant of

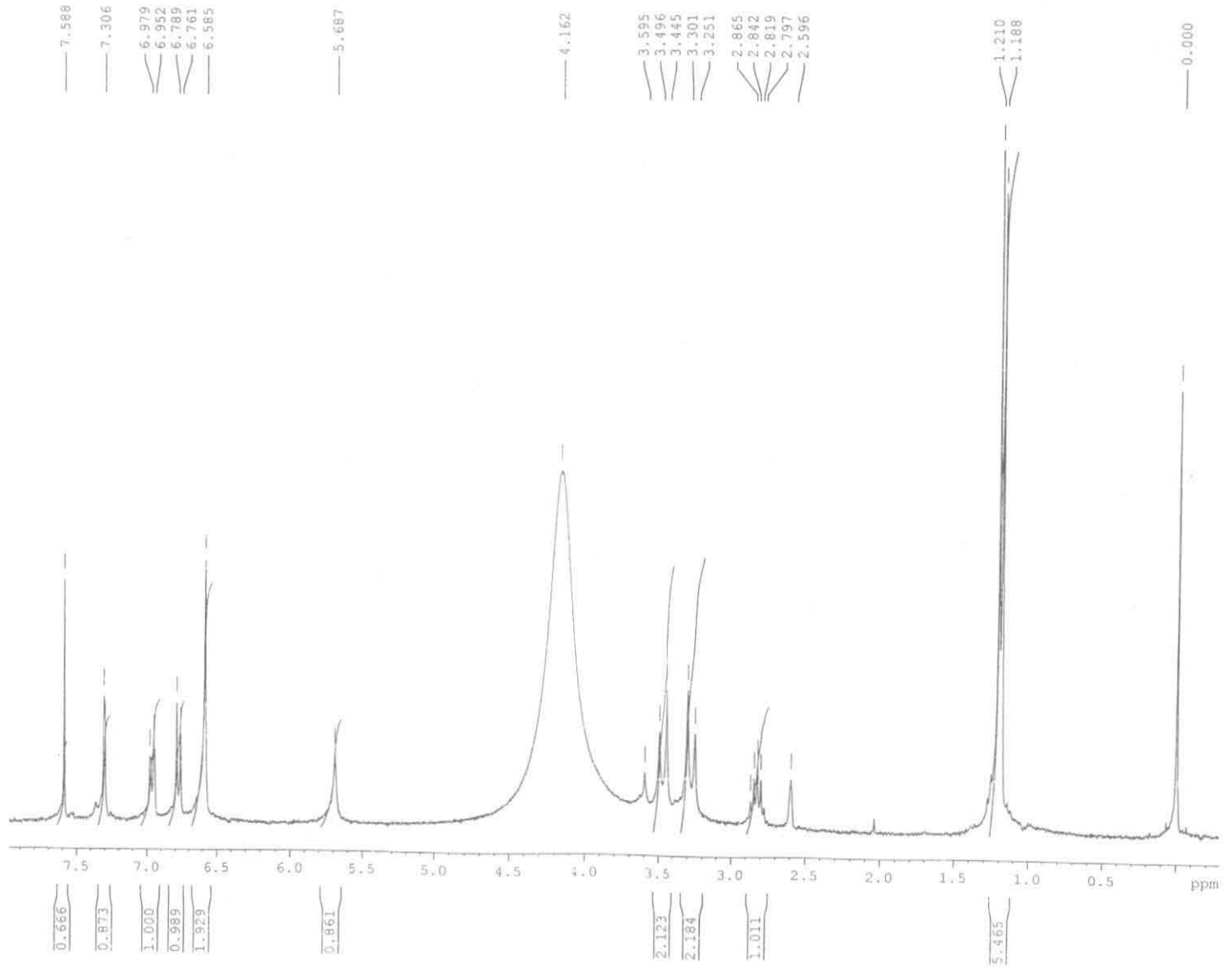



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Fig – 1 $^{1}$H NMR spectrum of 2e

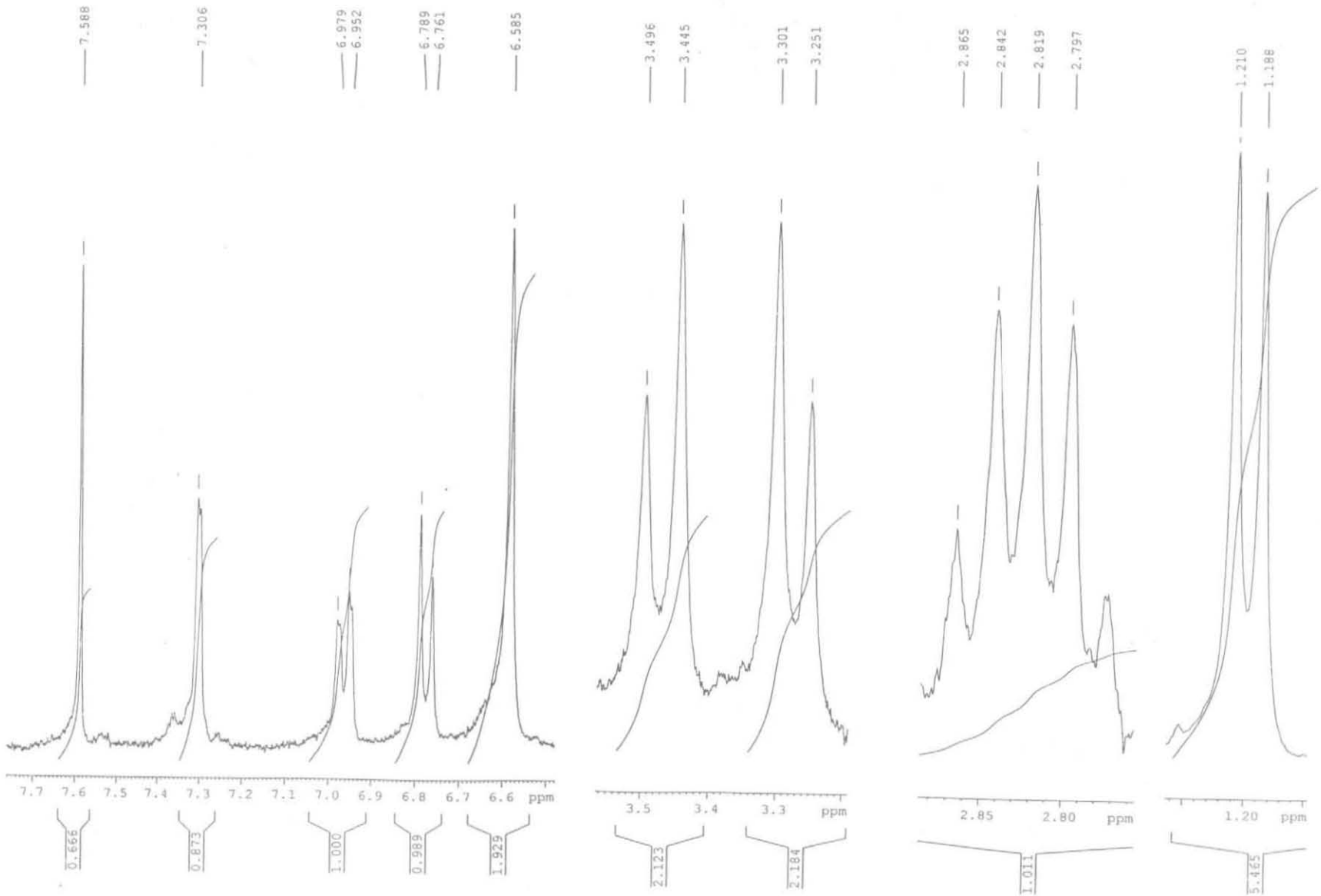



**Fig – 2 $^{1}$H NMR spectrum of 2e (Expanded)**

15 Hz. Singlets have been observed at 5.68, 6.58 and 7.31 ppm and a pair of doublets was seen at 6.96 and 6.77 ppm (J = 8.4 Hz). The C-13 NMR spectrum (**Fig - 3**) exhibits 13 signals. By the combined use of DEPT 90 (**Fig - 4**) and DEPT 135 (**Fig - 5**) experiments, the carbons at 33.1, 47.3, 117.4, 115.8, 125.6 and 126.6 are found to be methine carbons and the carbon at 34.4 ppm as methylene carbon and the carbon at 24.1 ppm as methyl carbon leaving the remaining carbons at 124.6, 136.6, 139.5, 152.0 and 172.0 ppm to be quaternary carbons. The H,H COSY (**Fig - 6**) CH COSY (**Fig - 7**) and HMBC (**Fig - 8**) connectivities are listed below:

H, H Connectivity:

3.27 ppm with 3.47 ppm; 2.82 ppm with 1.20 ppm 6.96 ppm with 6.77 ppm

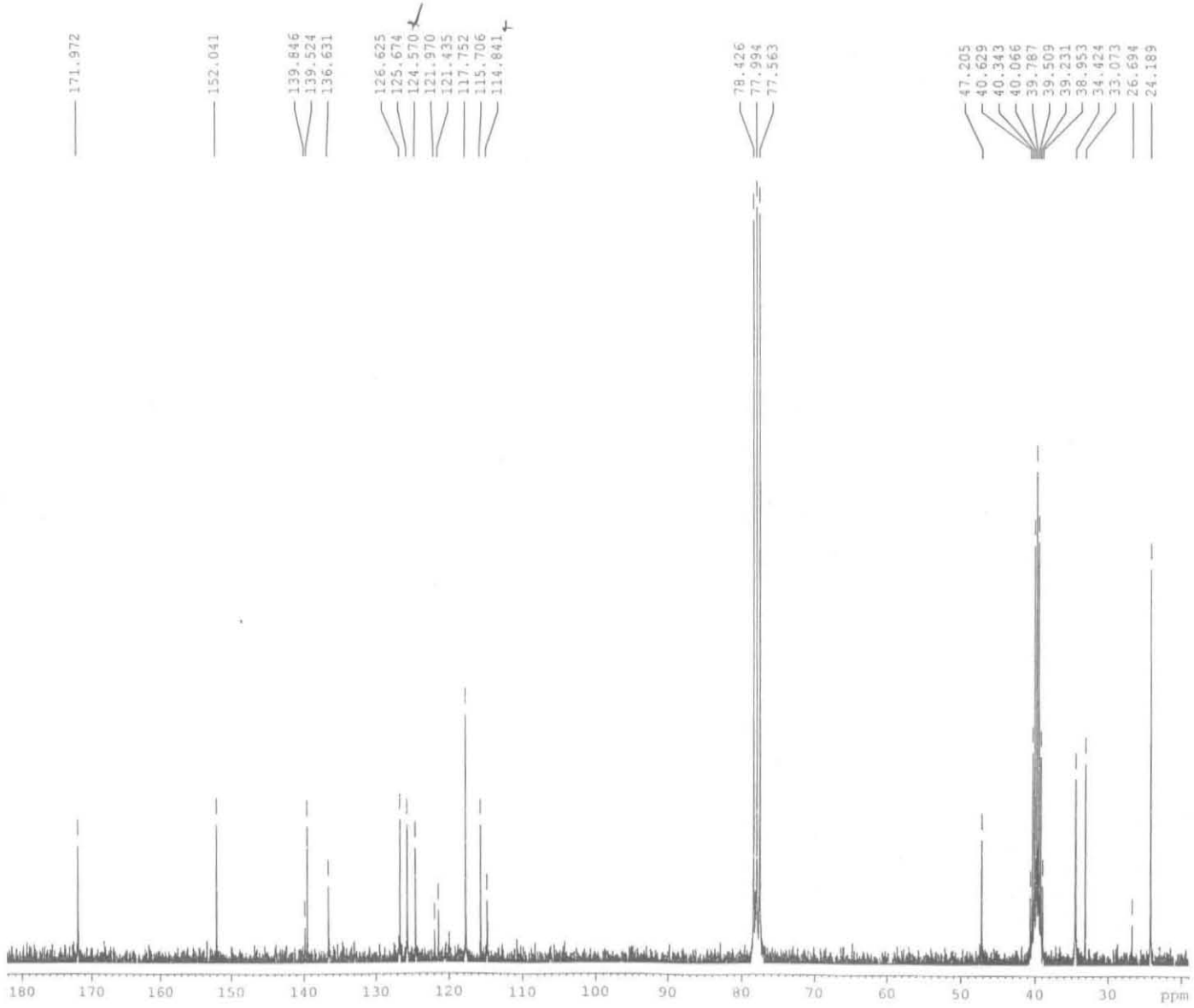
C, H connectivity:

33.1 ppm with 2.82 ppm; 47.3 ppm with 5.68 ppm 117.4 ppm with 6.58 ppm
115.8 ppm with 6.77 ppm 125.6 ppm with 7.31 ppm 126.6 with 6.96 ppm found
34.4 ppm with 3.27 and 3.47 ppm 24.1 ppm with 1.20 ppm

HMBC connectivity:

Hydrogen at 7.31 ppm has contours with carbons at 126.6, 47.3, 33.1 and 152.0 ppm
Hydrogen at 6.96 ppm has contours with carbons at 125.6 and 152.0 ppm
Hydrogen at 6.77 ppm has contours with carbons at 139.5 and 124.6 ppm
Hydrogen at 6.58 ppm has contours with carbons at 136.6 ppm
Hydrogens at 3.27 and 3.47 ppm have contours with carbons at 47.3 and 172.0 ppm
Hydrogen at 5.68 ppm has contours with carbons at 125.6 ppm
Hydrogen at 2.82 ppm has contours with carbons at 139.5 and 33.1 ppm
Hydrogen at 1.20 ppm has contours with carbons at 139.5 and 33.1 ppm

Based on the above data, complete assignment to all the carbons and hydrogens in this compound can be made as shown below (**Fig - 9**):

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FIDRES 0.274439 Hz
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SFO1 75.4752653 MHz

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F2 - Processing parameters
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Fig - 3 $^{13}$C NMR spectrum of 2e

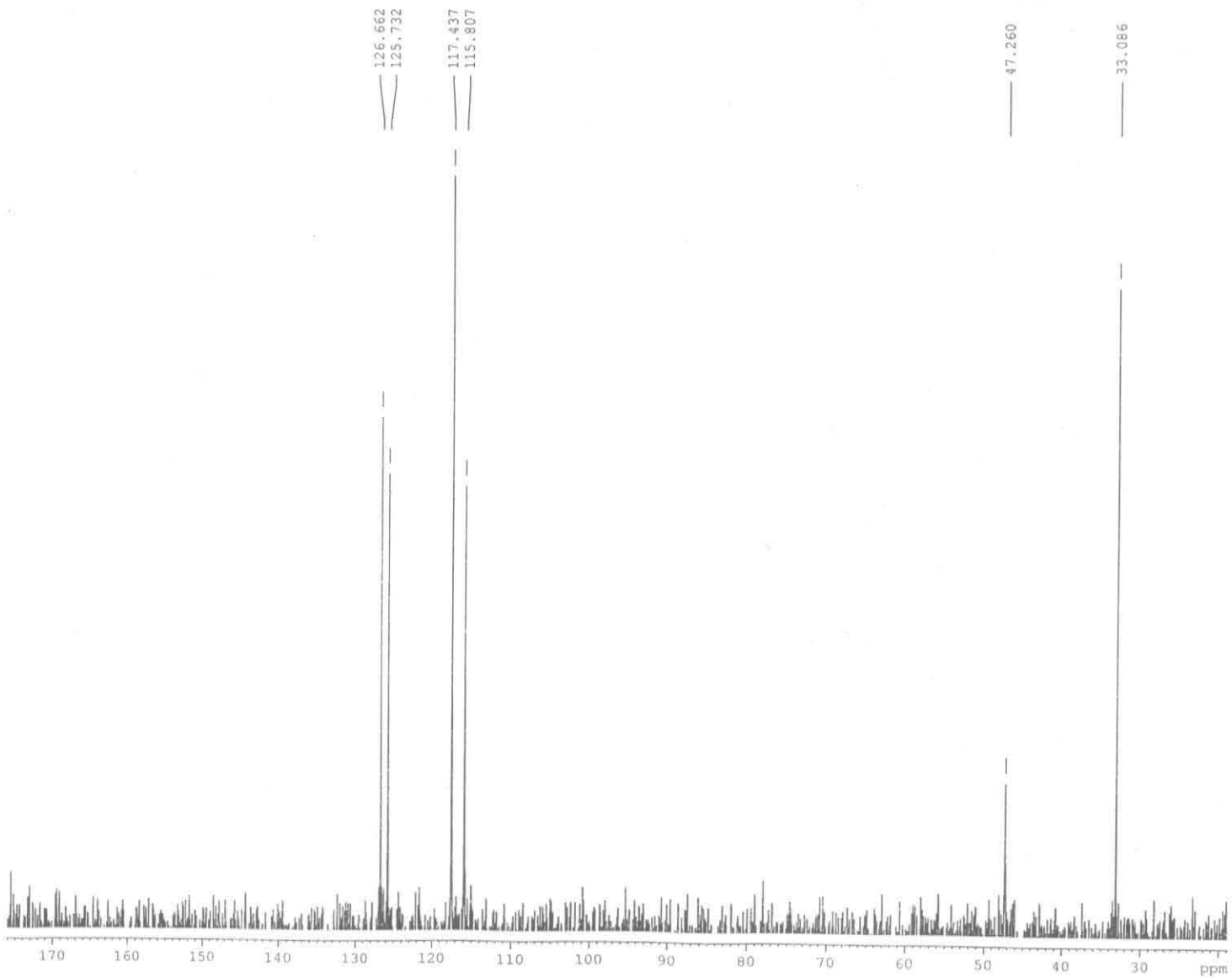


Fig - 4 DEPT 90 experiment of 2e

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d2                   0.00344828 sec
d12                  0.00002000 sec
DELTA                0.00000866 sec

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PCPD2                    80.00 usec
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PL12                     17.68 dB
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PC                         1.40
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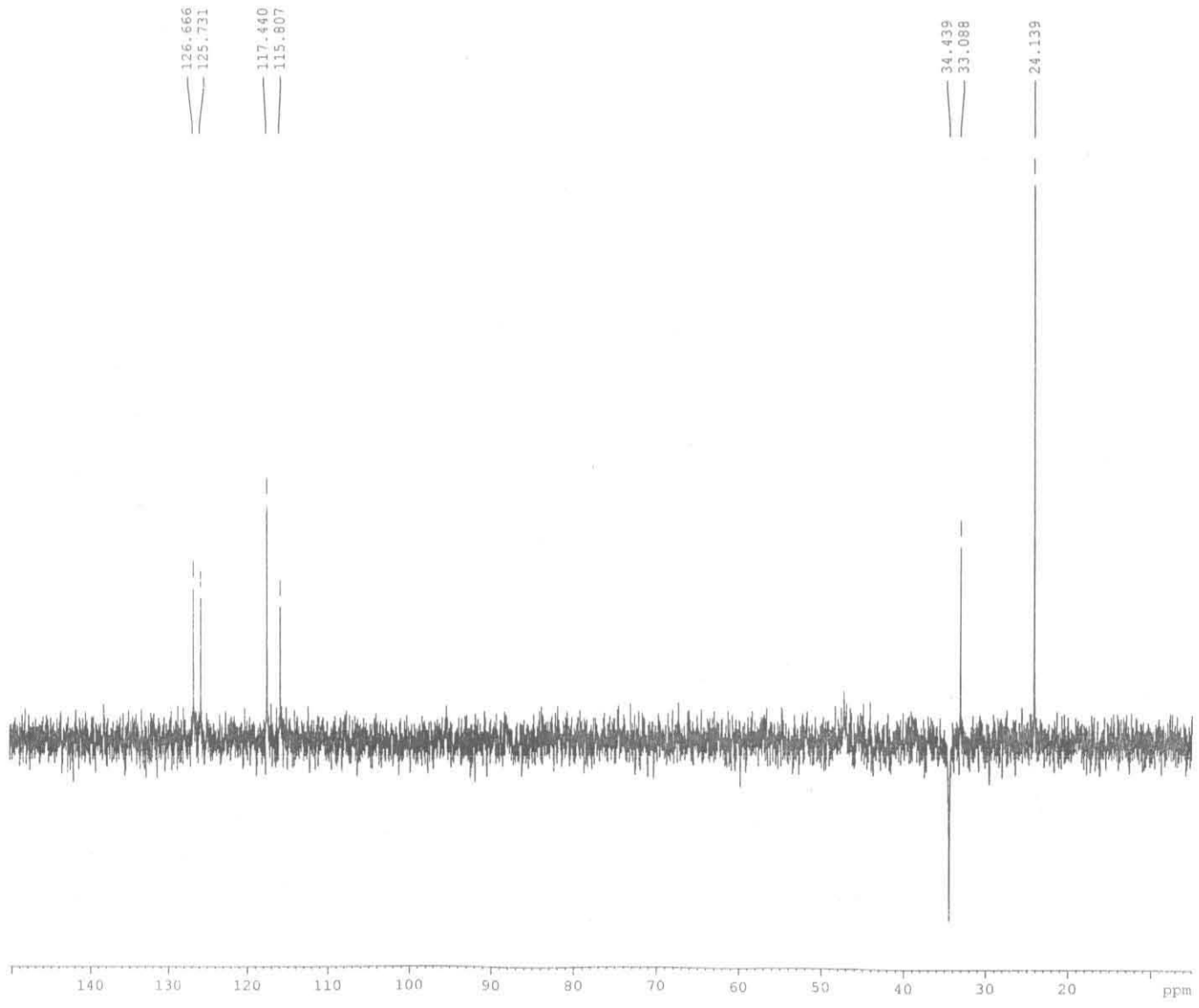


Current Data Parameters
NAME DI-11+Thio
EXPNO 53
PROCNO 1

F2 - Acquisition Parameters
Date_ 20021015
Time 16.16
INSTRUM av300
PROBHD 5 mm BBO BB-1H
PULPROG dept135
TD 65536
SOLVENT DMSO
NS 256
DS 4
SWH 17985.611 Hz
FIDRES 0.274439 Hz
AQ 1.8219508 sec
RG 16384
DW 27.800 usec
DE 6.00 usec
TE 300.0 K
CNST2 145.0000000
D1 2.00000000 sec
d2 0.00344828 sec
d12 0.00002000 sec
DELTA 0.00000866 sec

======== CHANNEL f1 ========
NUC1 13C
P1 6.80 usec
p2 13.60 usec
PL1 -4.00 dB
SFO1 75.4752653 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
P3 10.45 usec
p4 20.90 usec
PCPD2 80.00 usec
PL2 0.00 dB
PL12 17.68 dB
SFO2 300.1312005 MHz

F2 - Processing parameters
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SF 75.4677567 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

**Fig – 5 DEPT 135 experiment of 2e**

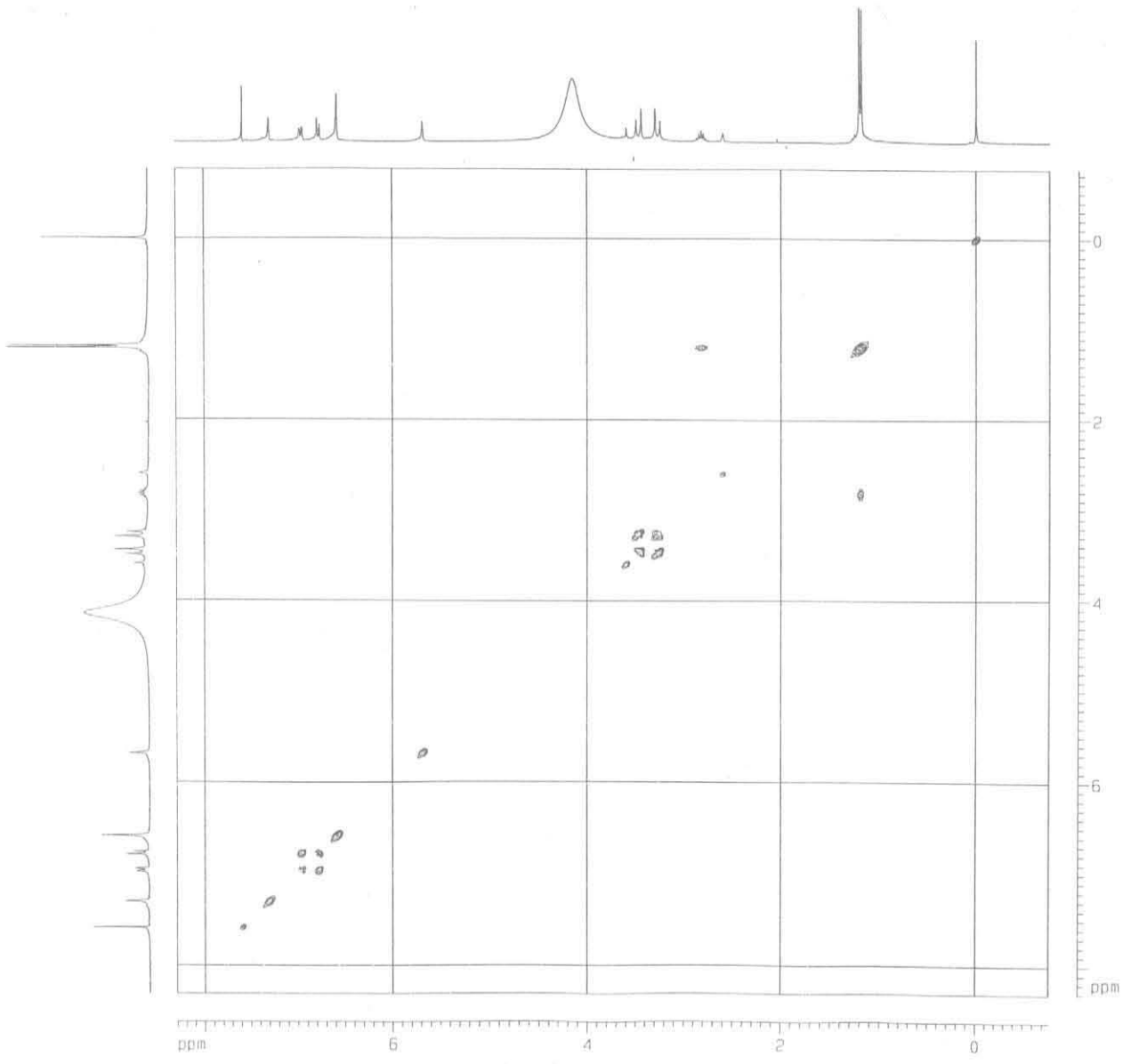


Fig – 6 H-H COSY experiment of 2e

```
                Current Data Parameters
NAME            DJ-E1+Thio
EXPNO                   44
PROCNO                   1

        F2 - Acquisition Parameters
Date_         20021015
Time              11.31
INSTRUM           av300
PROBHD  5 mm BBO BB-1H
PULPROG         cosygpqf
TD                2048
SOLVENT           DMSO
NS                   1
DS                   8
SWH           2723.312 Hz
FIDRES        1.329742 Hz
AQ            0.376062 sec
RG                645.1
DW              183.600 usec
DE                6.00 usec
TE                300.0 K
D0            0.00000300 sec
D1            1.36769795 sec
D13           0.00000400 sec
D16           0.00020000 sec
INO           0.00036720 sec
MCREST        0.00000000 sec
MCWRK         1.36769795 sec

        ======== CHANNEL f1 ========
NUC1                1H
P0                0.45 usec
P1               10.45 usec
PL1               0.00 dB
SFO1        300.1311050 MHz

        ====== GRADIENT CHANNEL =====
GPNAM1          SINE.100
GPNAM2          SINE.100
GPX1              0.00 %
GPX2              0.00 %
GPY1              0.00 %
GPY2              0.00 %
GPZ1             10.00 %
GPZ2             10.00 %
P16            1000.00 usec

        F1 - Acquisition parameters
NO0                  1
TD                 128
SFO1        300.1311 MHz
FIDRES       21.275871 Hz
SW            9.074 ppm

        F2 - Processing parameters
SI                1024
SF          300.1299733 MHz
WDW               SINE
SSB                  0
LB                0.00 Hz
GB                   0
PC                1.40

        F1 - Processing parameters
SI                1024
NC2                  0F
SF          300.1299733 MHz
WDW               SINE
SSB                  0
LB                0.00 Hz
GB                   0

        2D NMR plot parameters
CX2              15.00 cm
CX1              15.00 cm
F2PLO             8.308 ppm
F2LO           2493.35 Hz
F2PHI            -0.766 ppm
F2HI           -229.96 Hz
F1PLO             8.308 ppm
F1LO           2493.35 Hz
F1PHI            -0.766 ppm
F1HI           -229.96 Hz
F2PPMCM           0.60492 ppm/cm
F2HZCM          181.55411 Hz/cm
F1PPMCM           0.60492 ppm/cm
F1HZCM          181.55411 Hz/cm
```

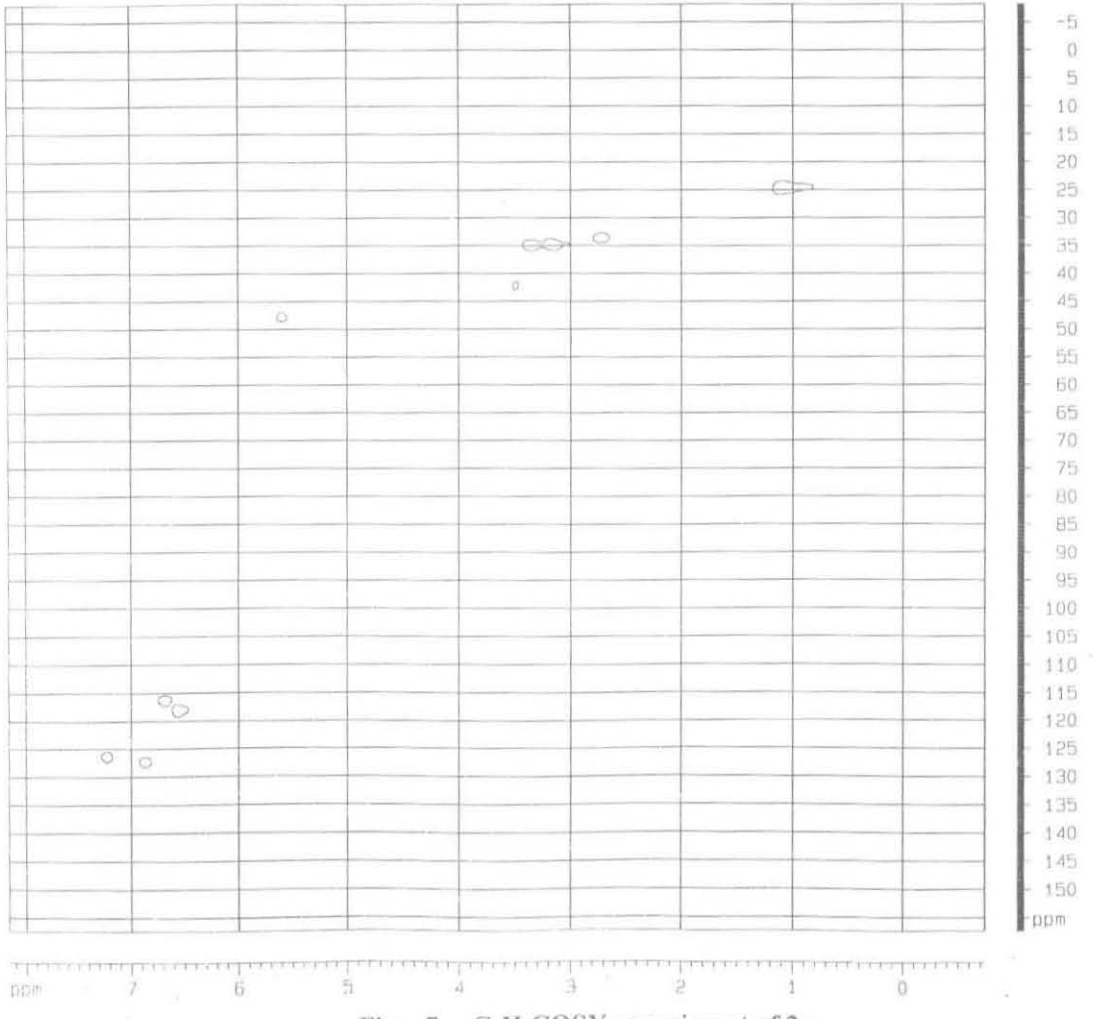


Fig – 7 C-H COSY experiment of 2e

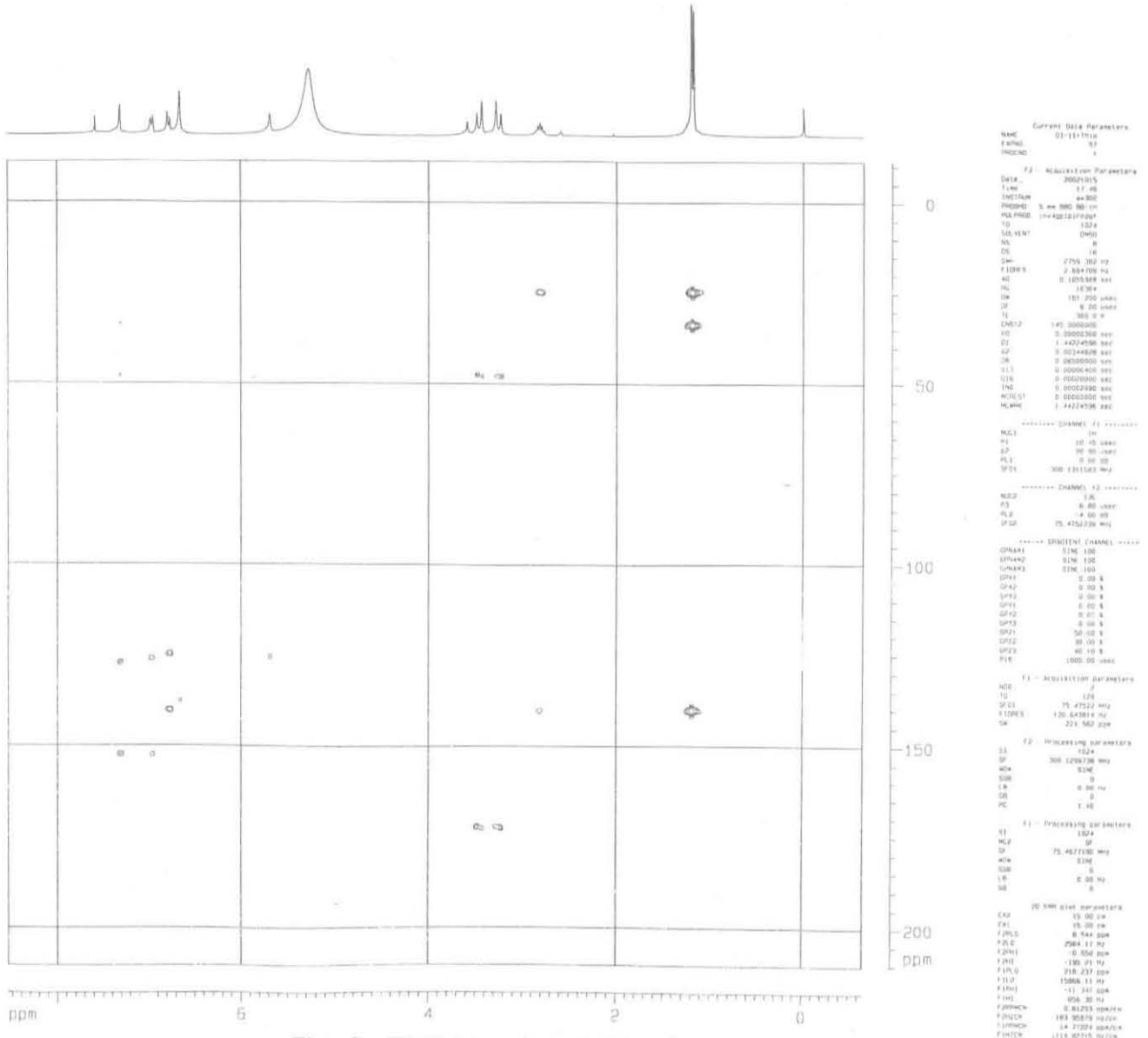


**Fig – 8 HMBC experiment of 2e**

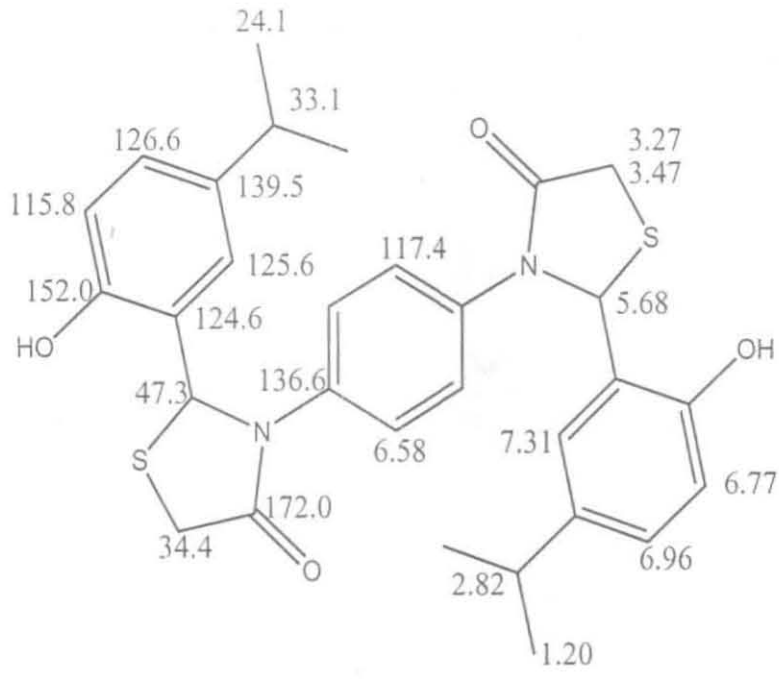


**Fig - 9**: NMR assignment for compound **2e**

Hydrogen bonding involved in these compounds, whether with nitrogen or sulphur, can not be ascertained as the NMR spectra recorded do not have any hydroxyl signal, probably exchanged with the trace amount of water present in DMSO-$d_6$.

The synthesized compounds **2a - 2e** have been tested for antifungal activities. The results are presented below.

Search for improved synthetic chemicals of fungistatic nature leads to the laboratory studies to select such chemicals before taking up the time consuming and costly field trials. Biological spectrum and sensitivity reaction of any fungi static chemical are two of such important criteria for evaluating the efficacy of the chemical compound (Fungicide). Fungicides with broader biological spectrum and high sensitiveness have the advantage over the narrower biological spectrum and low sensitivity reaction fungicides in their application for plant disease control. The laboratory studies have been undertaken to evaluate the efficacy of the synthesised compounds on the basis of their inhibitory range and sensitive reaction towards major phytopathogenic fungi *viz. Fusarium oxysporum* and *Rhizoctonia solani*.

Among the five compounds, **2a-2e,** checked for their antifungal efficiency, the compounds **2a** and **2b** showed antifungal activity against both the test fungi (**Table - 3**). Compound **2e** showed antibiosis against the fungi *R. solani* only. **2b** showed the efficient antibiosis against both the test fungi. The antifungal activity of these compounds is perhaps due to the presence of sulphur in these compounds. Hence, it can be used as fungicide against soil borne phytopathogens like *Fusarium* and *Rhizoctonia*, after confirming the activity *in vivo* by suitable field trials.

**Table – 3**: Biological spectrum and specificity of **2**

| Chemicals And | Average Radial Growth of Mycelium (mm) | | % Inhibition | |
|---|---|---|---|---|
| | **Fusaarium** *oxysporum* | **Rhizoctonia** *solani* | **Fusaarium** *oxysporum* | **Rhizoctonia** *Solani* |
| **2a** | | | | |
| 100 µg mL-1 | 87.00 ± 0.00 | 46.00 ± 5.29 | 0 | 47.12 ± 6.08 |
| 200 µg mL-1 | 66.66 ± 2.88 | 43.66 ± 5.29 | 23.36 ± 3.31 | 49.80 ± 3.31 |
| **2b** | | | | |
| 100 µg mL-1 | 46.66 ± 5.77 | 28.33 ± 7.63 | 51.85 ± 6.41 | 52.78 ± 12.72 |
| 200 µg mL-1 | 32.56 ± 3.31 | 20.25 ± 2.88 | 63.82 ± 6.41 | 66.25 ± 5.41 |
| **2c** | | | | |
| 100 µg mL-1 | 87 | 87 | 0 | 0 |
| 200 µg mL-1 | 87 | 87 | 0 | 0 |
| **2d** | | | | |
| 100 µg mL-1 | 87 | 87 | 0 | 0 |
| 200 µg mL-1 | 87 | 87 | 0 | 0 |
| **2e** | | | | |
| 100 µg mL-1 | 87.00 ± 0.00 | 66.66 ± 2.88 | 0 | 29.11 ± 3.31 |
| 200 µg mL-1 | 87.00 ± 0.00 | 54.00 ± 5.29 | 0 | 37.92 ± 6.07 |

± Standard Deviation

To the best of our knowledge, this is the first instance in which an organic molecule with two thiazolidine rings is being reported.

Apart from the above noted biological characteristics for the 1,3-thoazolidin-4-ones **2**, their chelating ability of **2** are also worth investigating, as there is an acidic phenolic hydroxyl and heteroatoms nitrogen and sulphur in the appropriate positions suitable for chelate formation. Work on this line is under progress in this laboratory.

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# Summary

## Summary

Marine natural products chemistry emerging as a multidisciplinary field drawing attention of chemists, along with pharmacologists and biologists is to explore compounds of novel features both in structure and biological activities. The recent trend of more biological screening along with structural identification of the compounds present in extracts of marine organisms shows that there is increased potential application of these secondary metabolites. The richness in marine bio diversity as the source of various biologically active compounds is now felt extensively.

Gulf of Mannar Marine Biosphere Reserve (GOMMBR) present in South East Indian Coastal area has the coastal bio diversity housing appreciable number of various marine organisms. Though the GOMMBR is rich in various flora and fauna, work on the chemical aspects of its organisms is not well explored. At present, reports on Alcyonarian corals of this region are coming, but reports on sponges and finfishes are very scarce.

This thesis describes the first chemical investigation of the marine organisms of Palk Bay, *viz.* sponges and a puffer fish. The organisms investigated in this thesis include five genera of sponges *viz. Siphonochalina* spp., *Cervicornia* spp., *Hippospongia* spp., *Hyrtios* spp. and *Spongia* spp. and puffer fish, *Tetraodon* spp. The analyses have been carried out using GC-MS and most of the compounds have been characterized by their mass spectra by comparison with the data of standard compounds.

The third chapter describes the chemical study of *Siphonochalina.* spp. Three fractions obtained have been analysed and nine compounds have been characterised from the first fraction, one each from the other two fractions.

The fourth chapter describes the identification of compounds from three fractions collected from *Cervicornia* spp. Eleven fatty acid methyl esters, eight free fatty acids and ten other compounds have been found to be present respectively in these fractions.

The fifth chapter deals with the chemical exploration of *Hippospongia* spp. isolating four fractions from the petroleum ether extract. There are nine compounds in first fraction, nine compounds in second fraction, two from the third and five from the last fraction. Different long chain hydrocarbons, long chain fatty acid methyl esters and steroids have been identified from these fractions.

The sixth chapter deals with the chemical exploration of *Hyrtios* spp. to get five fractions from the carbon tetrachloride extract. The number of compounds identified in these fractions are fifteen, one, two, three and three compounds respectively. Long chain fatty acid methyl esters, phthalyl derivative, long chain fatty alcohol and some steroids have been found in this organism.

The seventh chapter deals with the chemical exploration of *Spongia* spp. Seven fractions from carbon tetrachloride extract and three fractions from ethyl acetate extract have been obtained with this sponge. These seven fractions of the carbon tetrachloride extract have been analysed and found to contain one, twenty three, four, eight, three, two and one compound respectively. Of the three fractions of the ethyl acetate extract, the number of compounds identified are two, four and one respectively. The types of compounds present in this sponge are terpene, long chain fatty acid methyl esters, phthalyl derivative, free fatty acids, steroids and benzenoid derivatives. This sponge has been already been reported to be the storehouse of sesterterpenes, scalarane derived compounds, furano and thiazole containing compounds.

The eighth chapter deals with the chemical analysis of a puffer fish, *Tetraodon* spp. Two compounds have been isolated from the aqueous extract and they have been shown to be a cyclic amide and a *para* substituted aromatic compound with the help of spectral techniques.

The ninth chapter describes the synthesis, characterisation and evaluation for antifungal activity of some of thiazolidine derivatives. The syntheses reported involve microwave irradiation of the respective reactants. To the best of our knowledge, this is the first instance in which an organic molecule with two thiazolidine ring is being reported.